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CONTENTS

ILLUSTRATIONS

PLATES

TEXT-FIGURES

THE RELATIVE WEIGHT OF SHOOT AND ROOT OF DIFFERENT CROPS AND
ITS AGRICULTURAL SIGNIFICANCE IN RELATION TO THE AMOUNT
OF PHOSPHATE ADDED TO THE SOIL

NITROGEN METABOLISM OF SOYBEANS IN RELATION TO THE
SYMBIOTIC NITROGEN FIXATION PROCESS

EFFECT OF FERTILIZERS ON SOME NITROGENOUS AND OTHER CONSTITUENTS
OF THE COTTON PLANT AS SEPARATED BY ELECTRODIALYSIS
AT DIFFERENT STAGES OF GROWTH

NUTRIENT CONTENT AND BASE EXCHANGE PROPERTIES OF ORGANIC
LAYERS OF FOREST SOILS IN THE LAKE STATES REGION

A SIMPLE TYPE OF ELECTRICAL SALINOMETER FOR ESTIMATING SOLUBLE
SALTS IN SOILS AND IRRIGATION WATERS

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ADAPTING HIGH ANALYSIS AND CONCENTRATED FERTILIZERS TO COTTON SOILS

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The rapid development of the world nitrogen-industry following the World War and a corresponding advance in fertilizer technology resulted in the introduction of concentrated fertilizers in the period 1920–1930.

When concentrated fertilizers were first introduced, little was known regarding their efficiency. The United States Department of Agriculture and several coöperating state agricultural experiment stations initiated experiments with concentrated fertilizers for various crops. The objectives of these experiments were to determine the factors influencing the efficiency of concentrated fertilizers and to compare various concentrated fertilizers with standard strength mixtures. Experiments with these fertilizers for cotton were conducted in Virginia, North Carolina, South Carolina, and Georgia during the 12-year period, 1925–1936. The purpose of this paper is to summarize the results and to indicate some conclusions that have been reached.

DIFFERENCES BETWEEN STANDARD STRENGTH AND CONCENTRATED FERTILIZERS

The standard fertilizers used in these experiments were made from the older fertilizer materials and contained superphosphate, potassium sulfate, and potassium chloride, and equivalent amounts of nitrogen from ammonium sulfate, sodium nitrate, and cottonseed meal. The various concentrated fertilizers were made from the newer fertilizer salts, many of synthetic origin, and contained mono-ammonium phosphate, triple superphosphate, and potassium ammonium phosphate[1] as a source of phosphoric acid. Potash was derived from potassium sulfate, potassium nitrate, potassium chloride, and potassium ammonium phosphate. Nitrogen was derived from one or more of the following sources: mono-ammonium phosphate, urea, ammonium nitrate, potassium nitrate, ammonium chloride, and potassium ammonium phosphate. The phosphoric acid in both the standard and concentrated fertilizers was water soluble. Likewise, the potash in the two types of fertilizers was derived from essentially the same water-soluble sources.

Nitrogen in the concentrated fertilizers was completely water-soluble,

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[1] The potassium ammonium phosphate used in these experiments was made in the laboratory of the Bureau of Chemistry and Soils by W. H. Ross and A. R. Merze.

1

whereas one-third of the nitrogen in most of the standard fertilizers was from water-insoluble organic sources. All the standard strength fertilizers contained nitrate nitrogen, whereas many of the concentrated fertilizers contained only ammonium and soluble organic nitrogen.

Closely related to the source of nitrogen in the two types of fertilizers is the potential or equivalent acidity of the fertilizer. The standard fertilizers were formulated so that they were only slightly acid. Most of the concentrated fertilizers were moderately to strongly acid. Since many of the experiments were conducted on acid soils, this property may materially influence the results secured with the two types of fertilizers.

The standard fertilizer contained calcium and a small amount of magnesium and sulfates. The concentrated fertilizers made with ammonium phosphate were practically calcium- and magnesium-free mixtures. Sulfur was supplied by the potash salts. When made with triple super-phosphate, the concentrated fertilizers also contained calcium. Practically no magnesium was present.

The fertilizers may differ in their content of minor plant food elements, manganese, zinc, copper, and boron, but the extent of these differences is not known. The standard fertilizers may be higher in minor plant food elements than the concentrated fertilizer as they contain natural organic nitrogen sources. Ammonium phosphate, triple superphosphate, and potash salts, however, which were used in the concentrated fertilizers, may contain many of the minor plant food elements, as shown by Gaddum and Rogers (3).

The foregoing considerations indicate that source of nitrogen; equivalent acidity of fertilizer; content of secondary plant food elements, calcium, magnesium, and sulfur; and content of minor plant food elements may be the more important differences between the standard and concentrated fertilizers used in these experiments.

DEVELOPMENT OF THE WORK

In 1925, experiments of a preliminary nature were made on Marlboro fine sandy loam and Cecil sandy loam in North Carolina, in which two concentrated fertilizer mixtures, made in part from different materials, were compared with a standard 5–9–3 fertilizer. These experiments were planned to study the effects of source of materials and concentration of plant food. Based on these results, extensive experiments were planned using various sources and combinations of fertilizer salts. The concentrated fertilizers used in the earlier experiments contained 2.5 times the amount of nitrogen, phosphoric acid, and potash as the 5–9–3.

The experiments were made on soil types occurring in large areas and used extensively for cotton growing in the coastal plain and Piedmont sections of the southeastern cotton belt. Cotton was grown on the field the year preceding the inauguration of the experiment. At this time, growth and yields were noted to determine the uniformity of the soil. The experimental fields were divided, in most cases, into approximately $\frac{1}{10}$ acre plots.

SERIES 1: A COMPARISON OF A STANDARD AND SEVERAL CONCENTRATED FERTILIZERS

The results of a series of five experiments, conducted from one to six years, each at a different location and comparing a 5–9–3 fertilizer with eight different

TABLE 1

Results of experiments in series 1: Average yield (per acre) of seed cotton on two soils with a standard and various concentrated fertilizers

PLOT NO.	COMPOSITION OF FERTILIZER*	ACIDITY PER UNIT NITROGEN, LBS. CaCO₃	CECIL CLAY LOAM					MARLBORO SANDY LOAM		WEIGHTED AVERAGE OF ALL EXPERIMENTS†
			Expt. 1	Expt 2			Expt. 3	Expt. 4	Expt. 5	
			1927–1928	1928–1930	1931–1933	1928–1933	1928–1929	1926	1928	
			lbs.	lbs	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
1	Superphosphate, sodium nitrate, ammonium sulfate, cottonseed, tankage, potassium sulfate	25	990	904	1,367	1,136	1,047	1,688	1,446	1,121
2	Ammonium phosphate, ammonium nitrate, potassium nitrate	44	1,006	922	1,172	1,047	989	1,698	1,488	1,069
3	Ammonium phosphate, urea, potassium sulfate	52	933	895	1,229	1,062	993	1,538	1,452	1,061
4	Ammonium phosphate, ammonium chloride, potassium sulfate	107	891	847	1,113	980	783	1,304	1,500	975
5	Ammonium phosphate, ammonium sulfate, potassium sulfate	107	945	951	1,072	1,012	857	1,400	1,596	1,024
6	Ammonium phosphate, sodium nitrate, potassium sulfate	30	971	861	1,132	996	980	.....	1,596	1,043
7	Potassium ammonium phosphate, urea, potassium nitrate	...	961	822	1,151	986	772	.....	1,314	972
8	Potassium ammonium phosphate, ammonium sulfate, potassium nitrate	...	908	845	1,095	970	823	.....	1,482	978
9	Triple superphosphate, urea, potassium sulfate	36	966	852	1,125	988	962	1,540	1,410	1,017
10	No fertilizer		343	457	549	503	565	763	390	474

* Fertilizer on plot 1 was a 5–9–3 and was applied in the seed furrow and ridged 10 days before planting. Fertilizers on plots 2 to 9 were 2.5 times the concentration of that on plot 1 and were applied to give the same quantity of nitrogen, phosphoric acid, and potash.

† Experiment No. 4 not included in the average.

fertilizers that were 2.5 times as concentrated, are shown in table 1. The treatments at each location were in duplicate.

Experiment no. 1 at Charlotte, N. C., was carried on for two years. The data show that the 5–9–3 and six concentrated fertilizers gave somewhat similar yields. The yields from the 5–9–3 were slightly larger than five, and slightly less than one of the concentrated mixtures. Only two of the concentrated fertilizers were distinctly inferior to the 5–9–3 fertilizer.

Experiment no. 2 was conducted on Cecil clay loam at Youngsville, N. C., for six years. The data give the average for the first and second 3-year periods, as well as for the entire 6-year period. During the first 3 years, 1928–1930, two of the concentrated fertilizers produced slightly greater yields than the 5–9–3. None of the concentrated fertilizers was decidedly poorer than the standard. In the second 3-year period, however, the best concentrated fertilizer produced 138 pounds of seed cotton less than the 5–9–3. Nearly all of the other concentrated fertilizers produced 200 to 250 pounds less than the standard. This apparent change in the efficiency of concentrated fertilizers from the first to the second 3-year period may be significant. The good results secured during the first 3 years indicate that the concentrated fertilizers were equal to the 5–9–3 as a source of nitrogen, phosphoric acid, and potash. The poorer results during the last 3 years indicate that some secondary cumulative factors were limiting growth.

Experiment no. 3, conducted for 2 years at Griffin, Ga., showed that 5–9–3 was better than the concentrated fertilizers. Some of the concentrated fertilizers gave relatively poor results, but there is no apparent explanation for the difference in yields from the various mixtures, unless it can be attributed to a high degree of acidity, this being the most acid soil in the group.

A 1-year test on Marlboro sandy loam at Seaboard, N. C., experiment no. 4, indicates that a concentrated fertilizer of low equivalent acidity (plot 2) is as good as the 5–9–3, but that very acid fertilizers (plots 4 and 5) are decidedly inferior to the 5–9–3. The correlation between equivalent acidity of the fertilizer and yield is very good.

Another 1-year test, experiment no. 5 was conducted on Marlboro sandy loam at Wilson, N. C. Six of the eight concentrated fertilizers gave somewhat larger yields than the standard 5–9–3.

In two experiments preceding series 1 made in 1927 on Davidson clay at Lexington, N. C., and Marlboro sandy loam at Wilson, N. C., on ½-acre plots, harvested in 10 separate blocks, the effects of a concentrated and a standard fertilizer on germination, emergence of cotton, early growth, and yield was studied. The fertilizer mixtures were applied in the seed furrow by single-row distributors, having a forced feed and stirring screw, and then were bedded on with two furrows. Cotton was planted five days after the fertilizers were applied.

Records were made one month after the seeds were planted and before the cotton was chopped. Samples of soil were also taken from a section adjacent to the young plant roots, and their salt content was determined by the electric bridge method. The results are given in table 2.

The cotton on the heavy Davidson clay germinated well and made equal growth in the two fertilized plots. On the lighter Marlboro sandy loam, the cotton in the concentrated fertilizer plot did not germinate as well as that on the standard fertilizer plot nor did it grow as fast during the first month. In August, there was no apparent difference in growth from the two fertilizers on each soil. This condition is reflected in the yields, which are approximately the same.

The results of this series of experiments at all locations indicate that as sources of nitrogen, phosphoric acid, and potash, the concentrated fertilizers compare favorably with the 5–9–3; however, the yields from most of the concentrated mixtures are slightly lower in some cases and lower when an average of all the experiments is taken. The 5–9–3 proved more satisfactory

TABLE 2

Effect on cotton plants and yield of a standard and a concentrated fertilizer on two soils

DATA SECURED	DAVIDSON CLAY			MARLBORO SANDY LOAM		
	Standard* Fertilizer	Concentrated† Fertilizer	No Fertilizer	Standard* Fertilizer	Concentrated† Fertilizer	No Fertilizer
Seed germination (per cent) .	100	100	100	89	70	97
Average height of plants 1 month after seed was planted (inches)	2.85	2 80	2.42	3.47	2.28	2.37
Total soluble salts in soil (per cent)........	0.098	0.080	0.025	0.084	0.188	0.017
Yield of cotton (pounds per acre)........	1,222	1,240	492	1,426	1,442	725

* Standard fertilizer was 5–9–3 analysis and contained superphosphate, sodium nitrate, ammonium sulfate, natural organic nitrogen, and potassium sulfate, and was applied at the rate of 900 pounds per acre.

† Concentrated fertilizer contained mono-ammonium phosphate, urea, and potassium sulfate and was applied to give the same quantity of nitrogen, phosphoric acid, and potash.

on continued use on Cecil clay loam at Youngsville, possibly because of its lower equivalent acidity and higher content of calcium and magnesium. These properties may have given the 5–9–3 an advantage over the concentrated fertilizers even in short time tests, as in the experiment on Marlboro sandy loam at Seaboard, N. C.

The soils on which the experiments were made are acid, but their degree of acidity at the time the work was inaugurated did not vary widely. The changes in acidity that might have been due to the fertilizers were not determined. The soil of experiment 1 at Charlotte had a pH of 5.7; no. 2, at Youngsville, 5.3; no. 3, at Griffin, 5.0; no. 4, at Seaboard, 5.1; and no. 5, at Wilson, 5.6. Possibly the varying effects of the fertilizers on yield on the different soils could be attributed at least in part to acidity.

TABLE 3

Results of experiments in series 2: Influence of two standard and two concentrated fertilizers on the emergence of cotton

(Data are 7-year averages)

PLOT NO.	METHOD OF PLACEMENT	TIME OF PLANT-ING*	18-24-24†			6-8-8**		
			A	B	Av.	C	D	Av.
	Results on Onslow Sandy Loam, Holland, Va.							
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	Mixed in 6 in. band of soil, 3 in.	10	12	11	11.5	10	9	9.5
2	under seed	S	15	11	13.0	11	10	10.5
3	2 in. band of fertilizer, 3 in. under	10	12	17	14.5	12	8	10.0
4	seed	S	14	13	13.5	12	10	11.0
5	2 in. band of fertilizer to one side	10	10	8	9.0	9	7	8.0
6	and on level with seed	S	10	11	10.5	8	7	7.5
7	Mixed with soil and in contact	10	14	18	16.0	7	9	8.0
8	with seed	S	16	17	16.5	8	8	8.0
9	½ fertilizer applied as in no. 1, ½ as side dressing	..	6	8	7.0	7	3	5.0
10	Duplicate of no. 1	10	10	9	9.5	9	6	7.5
	Average of plots 1, 3, 5, 7	10	12	13.5	12.7	9.5	8.2	8.9
	Average of plots 2, 4, 6, 8	S	13.7	13.0	13.4	9.7	8.7	9.2
	Average of plots 1–10	..	11.9	12.3	12.1	9.3	7.5	8.5
	No fertilizer	..	7	8	7.5	6	4	5.0
	Results on Cecil Sandy Clay, Raleigh, N. C.††							
1	Mixed in 6 in. band of soil, 3 in.	10	15	17	16.0	11	10	10.5
2	under seed	S	19	18	18.5	12	11	11.5
3	2 in. band of fertilizer, 3 in. under	10	20	17	18.5	11	13	12.0
4	seed	S	16	17	16.5	13	12	12.5
5	2 in. band of fertilizer to one side	10	9	10	9.5	7	7	7.0
6	and on level with seed	S	9	11	10.0	9	7	8.0
7	Mixed with soil and in contact with	10	15	18	16.5	12	9	10.5
8	seed	S	16	17	16.5	11	12	11.5
9	½ fertilizer applied as in no. 1, ½ as side dressing	..	13	9	11.0	6	10	8.0

Header note: PERCENTAGE OF ROW AREA IN WHICH COTTON DID NOT COME UP

TABLE 3—*Concluded*

PLOT NO.	METHOD OF PLACEMENT	TIME OF PLANT-ING*	PERCENTAGE OF ROW AREA IN WHICH COTTON DID NOT COME UP					
			18–24–24†			6–8–8**		
			A	B	Av.	C	D	Av.

Results on Cecil Sandy Clay, Raleigh, N. C.††—*Concluded*

PLOT NO.	METHOD OF PLACEMENT	TIME OF PLANT-ING	A	B	Av.	C	D	Av.
			per cent	per cent	per cent	per cent	per cent	per cent
10	Duplicate of no. 1	10	18	16	17.0	8	9	8.5
	Average of plots 1, 3, 5, 7	10	14.8	15.5	15.1	10.2	9.8	10.0
	Average of plots 2, 4, 6, 8	S	15	15.7	15.4	11.2	10.5	10.9
	Average of plots 1–10	..	15	15	15.0	10	10	10.0

* 10—planted 10 days after fertilizer was applied; S—planted at time of fertilizer application.

† A contains ammonium phosphate, ammonium nitrate, and potassium sulfate. Urea substituted for ammonium nitrate in B. Application 266 lbs. per acre.

** C contains superphosphate, sodium nitrate, potassium sulfate. D contains equal amounts of nitrogen from sodium nitrate, ammonium sulfate, and cottonseed meal. Application 800 lbs. per acre.

†† 18–24–12 and 6–8–4 used.

SERIES 2: A COMPARISON OF STANDARD AND CONCENTRATED FERTILIZERS WITH DIFFERENT METHODS OF PLACEMENT

The results of a series of experiments started in 1928 at the Virginia Experiment Station, Holland, Va., and at the Central Experiment Station, Raleigh, N. C., in which two concentrated and two standard fertilizers were applied at different times and at different locations with respect to the position of the seed are given in tables 3, 4, and 5. The treatments were in duplicate.

The data of table 3 show that on Onslow sandy loam at Holland there were slight variations in the percentage of cotton which did not come up in the different fertilizer plots, but the grades of fertilizer and method or time of application did not greatly influence the emergence of cotton. An average of all the placements shows that 3.5 per cent less cotton came up when concentrated fertilizers were used than when standard fertilizers were used. When the fertilizer was mixed in the soil and in contact with the seed, or when it was placed in narrow bands below the seed, there was a more favorable emergence of plants from the standard than from concentrated fertilizers. When fertilizers were placed to the side of the seed, there was no significant difference. With the concentrated fertilizers, cotton failed to come up on 4.5 per cent more area than on the no-fertilizer plot. The corresponding figure for the standard fertilizer was 3.5 per cent.

On Cecil sandy clay at Raleigh, N. C., the emergence of cotton was about 5 per cent better with standard than with concentrated fertilizers. Emergence was just as good when the seed was planted the same day the fertilizer was

TABLE 4

Results of experiments in series 2: Influence of two standard and two concentrated fertilizers on the salt content of the soil solution

(Data are 7-year averages)

PLOT NO.	METHOD OF PLACEMENT	TIME OF PLANT-ING	SALTS IN SOIL OF THE PLANT'S ROOT ZONE* (10 DAYS AFTER EMERGENCE)					
			18–24–24*			6–8–8*		
			A	B	Av.	C	D	Av.
Results on Onslow Sandy Loam, Holland, Va.								
			p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1	Mixed in 6 in. band of soil, 3 in.	10	445	...	..	843	734	789
2	under seed	S	777	...	...	1,044	656	850
3	2 in. band of fertilizer, 3 in. under	10	706	...	...	683	675	679
4	seed	S	951	...	...	1,027	728	877
5	2 in. band of fertilizer to one side	10	597	...	...	456	491	474
6	and on level with seed	S	744	...	...	656	521	588
7	Mixed with soil and in contact with	10	609	...	...	961	853	907
8	seed	S	768	.	...	1,032	794	913
9	½ fertilizer applied as no. 1, ½ as side dressing	10	357	...	...	553	417	485
	Average of plots 1, 3, 5, 7	10	589	...	...	736	688	712
	Average of plots 2, 4, 6, 8	S	810	...	...	940	675	807
	Average of plots 1–9	..	661	...	...	806	652	729
Results on Cecil Sandy Loam, Raleigh, N. C.								
			p p.m.	*p.p.m.*	*p.p m.*	*p.p.m.*	*p p m.*	*p.p m.*
1	Mixed in 6 in. band of soil, 3 in.	10	435	475	455	665	760	713
2	under seed	S	385	580	483	635	905	770
3	2 in. band of fertilizer, 3 in. under	10	460	410	235	640	335	487
4	seed	S	585	330	457	490	545	513
5	2 in. band of fertilizer to one side	10	195	280	238	180	645	412
6	and on level with seed	S	385	195	290	580	425	503
7	Mixed with soil and in contact	10	545	755	650	295	670	482
8	with seed	S	320	555	437	565	740	653
	Average of plots 1, 3, 5, 7	10	409	480	395	445	603	524
	Average of plots, 2, 4, 6, 8	S	419	415	417	568	654	611
	Average of plots 1–8	..	414	448	431	506	628	567

' Details of the fertilizer composition are given in footnotes to table 3.

applied as when planting was delayed 10 days. When applied under the seed or mixed with the soil in contact with the seed, the concentrated fertilizers reduced emergence more than the standard fertilizers. The data indicate that emergence of cotton was slightly better with both fertilizers when the fertilizer was applied two inches to one side and on the level with the seed, or when one-half of the fertilizer was applied at planting and one-half as a side dressing.

The influence of method of placement of the fertilizer on the salt content of the soil solution in the fertilized zone is shown by the data in table 4. In securing these data, soil samples were taken from the region of the root zone of the young plants about 10 days after their emergence from the ground.

At Holland, Va., concentrated fertilizer A produced an average concentration of salts almost identical with that produced by standard fertilizer B, which derived its nitrogen from three sources. Standard fertilizer A, with all of the nitrogen from nitrate of soda, produced a higher salt content in the soil around the roots of plants than either of the other fertilizers. The results on Cecil sandy clay at Raleigh were somewhat different in that both concentrated fertilizers produced a lower salt content than either of the standard fertilizers.

The yield of seed cotton obtained in the experiments is given in table 5. On Onslow sandy loam at Holland, Va., neither the method of placement nor the time of planting, with respect to making the fertilizer application, materially influenced the yield of seed cotton obtained with either of the four fertilizers. Of the four fertilizers, best results were obtained with 8–24–24 A and the poorest results with 8–24–24 B. Fertilizer 6–8–8 D, in which half of the nitrogen was from natural organic sources, produced slightly better yields than 6–8–8 C, where all the nitrogen was derived from sodium nitrate. The Onslow sandy loam on which the experiment was made, as indicated by the yields from the untreated plots, was in a high state of fertility. This was due in part to the growing and incorporating of vegetation from green manure crops the year preceding the inauguration of the experiment and the growing of clover as a winter green manure crop while the experiment was in progress. The yields as a whole from the various fertilizers vary but little, and the differences are probably not very significant.

The results on Cecil sandy clay at Raleigh were materially different in several respects from those at Holland. Both fertilizers gave best results when placed in a 2-inch band to one side of, and on the level with, the seed. Placement, however, made more difference with the concentrated than with the standard fertilizer. Time of planting with respect to time of making the fertilizer application did not materially affect yields obtained with either the standard or the concentrated fertilizer.

The 6–8–4 C fertilizer produced the highest average yield. The lowest yield was obtained with 12–24–8 A, but both concentrated fertilizers were decidedly inferior to either of the standard fertilizers. The explanation for the observed difference may be more evident from a study of the data in table 6, which shows the influence of time on the efficiency of the different fertilizers at both Holland and Raleigh.

TABLE 5

Results of experiments in series 2: Influence of method of placement on yield (per acre) of seed cotton obtained with standard and high analysis fertilizers

(Data are 7-year averages)

PLOT NO.	METHOD OF PLACEMENT	TIME OF PLANT-ING	18-24-24*			6-8-8*		
			A	B	Av.	C	D	Av.
	Results on Onslow Sandy Loam, Holland, Va.							
			lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
1	Mixed in 6 in. band of soil, 3 in.	10	1,718	1,523	1,621	1,492	1,694	1,593
2	under seed	S	1,622	1,636	1,629	1,558	1,679	1,619
3	2 in. band of fertilizer 3 in. under	10	1,722	1,528	1,625	1,615	1,661	1,638
4	seed	S	1,695	1,503	1,599	1,562	1,576	1,569
5	2 in. band of fertilizer to one side	10	1,666	1,501	1,584	1,556	1,606	1,581
6	and on level with seed	S	1,643	1,516	1,580	1,578	1,586	1,582
7	Mixed with soil and in contact with	10	1,558	1,443	1,501	1,534	1,626	1,580
8	seed	S	1,675	1,514	1,595	1,540	1,623	1,582
9	½ fertilizer applied as no. 1, ½ as side dressing	..	1,647	1,506	1,577	1,556	1,501	1,529
10	Duplicate of no. 1	10	1,774	1,508	1,641	1,499	1,567	1,533
	Average of plots 1, 3, 5, 7	10	1,666	1,499	1,583	1,549	1,647	1,598
	Average of plots 2, 4, 6, 8	S	1,659	1,542	1,601	1,559	1,616	1,588
	Average of plots 1–10		1,672	1,518	1,595	1,549	1,612	1,580
	No fertilizer		1,262	1,195	1,228	1,165	1,115	1,140
	Results on Cecil Sandy Clay, Raleigh, N. C.							
			18-24-12			6-8-4		
			lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
1	Mixed in 6 in. band of soil, 3 in.	10	802	686	744	988	925	956
2	under seed	S	751	650	700	992	937	964
3	2 in. band of fertilizer 3 in. under	10	700	619	658	1,031	937	984
4	seed	S	732	665	699	1,072	912	992
5	2 in. band of fertilizer to one side	10	796	881	839	1,099	949	1,024
6	and on level with seed	S	735	864	800	1,068	902	985
7	Mixed with soil and in contact	10	648	719	684	939	844	892
8	with seed	S	612	727	670	859	788	824
9	½ fertilizer applied as no. 1, ½ as side dressing	..	717	844	780	897	782	840
10	Duplicate of no. 1	10	785	669	727	980	854	917
	Average of plots 1, 3, 5, 7	10	737	726	731	1,014	914	964
	Average of plots 2, 4, 6, 8	S	708	727	717	998	885	941
	Average of plots 1–10	..	728	732	724	993	883	937

* Details of the fertilizer composition are given in footnotes to table 3.

On the comparatively productive Onslow sandy loam at Holland, all four fertilizers produced somewhat better yields during the last three years than during the first four years of the experiment. It seems significant that, as compared to standard fertilizers, the concentrated fertilizers were just as efficient during the last three years as during the first four years of the experiment.

On the Cecil sandy clay in the Raleigh experiment, the concentrated fertilizer produced on the average only 112 pounds less seed cotton than the standard fertilizer, during the first four years. During the last three years, however,

TABLE 6

Results of experiments in series 2: Influence of duration of experiment on yield of seed cotton obtained with standard and concentrated fertilizers

FERTILIZER	AV. YIELD OF SEED COTTON (PER ACRE)		
	1928–1931	1932–1934	1928–1934
Results on Onslow Sandy Loam, Holland, Va.			
	lbs.	*lbs.*	*lbs.*
6–8–8 C	1,527	1,598	1,558
6–8–8 D	1,609	1,619	1,612
Average for 6–8–8	1,567	1,609	1,585
18–24–24 A	1,602	1,842	1,676
18–24–24 B	1,491	1,553	1,518
Average for 18–24–24	1,522	1,698	1,597
Results on Cecil Sandy Loam, Raleigh, N. C.			
	lbs.	*lbs.*	*lbs*
6–8–4 C	1,020	957	993
6–8–4 D	936	809	881
Average for 6–8–4	981	883	937
18–24–12 A	876	507	718
18–24–12 B	862	559	732
Average for 18–24–12	869	535	725

the standard fertilizer averaged 348 pounds seed cotton more than the concentrated fertilizers. This indicates that the efficiency of the concentrated fertilizers was decreasing with time. This is probably due to the fact that the concentrated fertilizers were acidic and practically calcium- and magnesium-free. The importance of the acidity factor is indicated by a comparison of the two 6–8–4 fertilizers. C is slightly basic whereas D is slightly acid. C outyielded D by 84 pounds during the first 4 years, whereas the difference increased to 149 pounds during the last 3 years. The increased difference is presumably due to the cumulative effect of the acidic fertilizer.

The different results secured at Holland and Raleigh may be readily ex-

plained on the basis of differences in the two soils.   The Onslow sandy loam at Holland is rather fertile and contains a fair amount of organic matter.   Its fertility is indicated by the fact that the no-fertilizer plots average approximately 1,200 pounds of seed cotton.   That is 200 pounds more than the best of the fertilized plots on the Cecil sandy clay at Raleigh.   The latter soil was in a very low state of fertility, as indicated by the yields.   Complete data on soil reaction were not obtained.   The Raleigh soil is very acid, whereas the Holland soil is only moderately acid.   In 1931, the average pH of the 12–24–8 B plots at Raleigh was 4.2, whereas in 1934, the reaction of the various plots at Holland averaged about pH 5.5.

In a second group of experiments in this series, another phase of the fertilizer placement study was begun in 1930 and continued for four years on four soil types in South Carolina[2] and North Carolina (1, 2, 4 to 9).   One single-strength and one double-strength fertilizer, made from standard fertilizer materials and containing calcium and magnesium, were used and applied at the rate of 800 and 400 pounds per acre, respectively, simultaneously with planting.   Three fertilizer placements were used.   These were replicated six times and the experiments were conducted for four years.   The same field for each soil type was not used each year.   The data are given in table 7.

Best stands and largest yields resulted from side placement of both the standard and double-strength fertilizer on all the soils, except in the case of the single-strength fertilizer on the heavy Cecil clay loam and the double-strength fertilizer on the Norfolk sandy loam.   This placement was generally more efficient than was placing in narrow bands below the seed or mixing with the soil below the seed.   This is in keeping with results of other experiments reported (1, 2).

A comparison of results obtained on the four soil types where 800 pounds of 4–8–4 were used with results of corresponding placements of 400 pounds of 8–16–8 generally shows no very marked difference in emergence of plants or in yield.   The double-strength fertilizer, on the Norfolk soil at Florence, S. C., with all placements, and on Norfolk soil at Rocky Mount, N. C., and Cecil soil at Clemson College, S. C., with two placements, gave better emergence of plants and larger yield than the standard fertilizer.

In 1931, the concentration of soluble salts in the seed zone was determined several times for each treatment, soon after planting the South Carolina experiments, by A. L. Mehring of the Bureau of Chemistry and Soils.   These results were previously reported (2).   Similar determinations were made in connection with the North Carolina experiment in 1933.   A knowledge of the quantity of

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[2] The experiments in South Carolina were conducted cooperatively with the South Carolina Agricultural Experiment Station on their substations at Columbia, Florence, and at Clemson College.   Dr. H. P. Cooper directed the experiment at Clemson College, and Dr. J. E. Adams, G. H. Serviss, and A. B. Bowen, of the Bureau of Plant Industry, were responsible for the agronomic data in the Columbia and Florence tests.   The machine placement work was under the direction of G. A. Cumings, of the Bureau of Agricultural Engineering.

soluble fertilizer which remained in the different soils, where it was placed, and how much came in contact during the germination period may be helpful in explaining the effect of different placements on the crop   It appears in these

TABLE 7

Results of experiments in second group of series 2: Effects of single-strength and double-strength fertilizer made from same standard materials on emergence of cotton plants and yield on four soils

(Data are 4-year Averages—1930–1933)

FERTILIZER PLACEMENT	SINGLE STRENGTH FERTI-LIZER, 4–8–4–800 LBS.		DOUBLE STRENGTH FERTI-LIZER, 8–16–8–400 LBS.	
	Plants emerging 50 ft. area	Yield per acre	Plants emerging 50 ft. area	Yield per acre
Norfolk coarse sand, Columbia, South Carolina				
	no.	*lbs.*	*no.*	*lbs.*
1.75 in. band, 2 in. below seed	97	346	146	466
2.25 in. to each side, 2 in. below seed level	397	616	385	577
Mixed with soil below seed	212	535	290	488
No fertilizer	395	197	405	169
Norfolk very fine sandy loam, Florence, S. C.				
	no	*lbs*	*no.*	*lbs.*
1.75 in. band, 2 in. below seed	112	1.074	133	1,093
2.25 in. to each side, 2 in. below seed level	330	1,490	342	1,772
Mixed with soil below seed	187	1,399	205	1,408
No fertilizer	346	1,341	343	1,374
Cecil sandy clay loam, Clemson College, S. C.				
	no	*lbs.*	*no.*	*lbs.*
1.75 in. band, 2 in. below seed	344	1,043	385	981
2.25 in. to each side, 2 in. below seed level	436	928	446	1,061
Mixed with soil below seed	318	1,022	422	933
No fertilizer	450	653	446	786
Norfolk sandy loam, Rocky Mt., N. C.				
	no.	*lbs.*	*no.*	*lbs.*
1.75 in. band, 2 in. below seed	153	1,182	170	1,380
2.25 in. to each side, 2 in. below seed level	314	1,594	298	1,570
Mixed with soil below seed	161	1,454	261	1,638
No fertilizer	277	971	...	.....

experiments that no salt was carried into contact with the seed from side placement when either fertilizer was used.   With other placements, salt was carried into the seed zone from applications of either fertilizer.   In all the soils, the quantity was greater when fertilizer of standard strength was used.

The degree of delay in germination and emergence of plants bore a close relationship to the amount of soluble salts carried into contact with the seed.

The results of the second series of experiments on six soils indicate that concentrated fertilizers have approximately the same influence on salt content of the soil as do standard grade fertilizers and do not unfavorably affect the stand, especially when properly applied. These studies further indicate that standard and double-strength fertilizers, containing calcium and magnesium, in addition to nitrogen, phosphoric acid, and potash, may be equally effective on some of the principal soil types of the cotton belt; and that salt concentration in the soil, which could have detrimental results for young plants, did not vary widely as the result of using the standard and double-strength fertilizer.

SERIES 3: THE INFLUENCE OF SUPPLEMENTS OF STANDARD FERTILIZER MATERIALS ON THE EFFICIENCY OF CONCENTRATED FERTILIZERS

In 1929, an experiment was started on Norfolk sandy loam at Goldsboro, N. C., to determine the influence of supplements of standard fertilizer materials and lime on the efficiency of concentrated fertilizers. The fertilizer treatments are detailed in table 8. These were repeated four times. Each fertilizer was applied in two ways, all before planting, and one half before planting and one half as a side dressing. The two methods of application gave almost identical results, so the yields for individual fertilizers are not shown. The recorded plot yields are the average resulting from the two methods of application. These data show that there is no advantage in splitting the fertilizer application. This, and the results of the experiments in series 2, definitely indicate that the best and most economical way of applying the fertilizer is in a band to one side of the seed at the time of planting.

The data of table 8 show that the various concentrated fertilizers, with one exception, produced a higher salt content of the soil solution than the standard 6–8–4. The differences were not large and probably were unimportant. The emergence of cotton was slightly better on the plot using standard 6–8–4, but in all cases good stands were obtained.

There is a fair correlation between the equivalent acidity of the fertilizer and the average pH value of the soil. The 6–8–4 plot was the least acid of all fertilized plots, except where limestone was applied as a supplement. Concentrated fertilizers used on plots 2, 3, and 4 were less acid than those used on 5, 6, and 7. Likewise, the average pH values of plots 5, 6, and 7 were lower than on plots 2, 3, and 4. The limestone supplement decreased the acidity of the soil from pH 5.06 on plot 5 to pH 6.05 on plot 8.

During the first three years, the yields from all fertilized plots were almost the same. The maximum variation was only 80 pounds of seed cotton. The supplements of inorganic-nitrogen and organic-nitrogen fertilizer materals apparently did not affect the efficiency of the concentrated fertilizer. Limestone apparently had a slight beneficial effect. The yields from concentrated fertilizers compare favorably with yields from the standard 6–8–4 fertilizer.

TABLE 8

*Influence of concentrated fertilizers plus supplements of standard fertilizer materials on soil reaction, salt content, and yield of seed cotton on Norfolk sandy loam**

PLOT NO.	PRINCIPAL INGREDIENTS	SUPPLEMENTARY†† MATERIALS	ACIDITY OF FERT. PER UNIT N	AVERAGE pH OF SOIL	SOLUBLE SALTS IN SOIL	AREA WITH NO PLANT EMERGENCE	AV. YIELD OF SEED COTTON		
							1929–1931	1932–1934	1929–1934
			lbs.	pH	p.p.m.	per cent	lbs.	lbs.	lbs.
1†	Superphosphate, sodium nitrate, ammonium sulfate, cottonseed meal, tankage, potassium sulfate	None	24	5.61	367	6.5	1,385	1,482	1,434
2**	Ammonium phosphate, ammonium nitrate, potassium nitrate	None	29	5.27	519	8.5	1,383	1,312	1,348
3	Same as No. 2	Sodium nitrate, ammonium sulfate	29	5.38	475	9.5	1,369	1,307	1,337
4	Same as No. 2	Cottonseed meal, tankage	26	5.57	409	6.5	1,348	1,384	1,366
5	Ammonium phosphate, urea, potassium sulfate	None	44	5.06	453	10.5	1,346	1,266	1,306
6	Same as No. 5	Sodium nitrate, ammonium sulfate	44	5.25	463	10.0	1,396	1,320	1,358
7	Same as No. 5	Cottonseed meal, tankage	41	5.45	353	9.5	1,349	1,311	1,330
8	Same as No. 5	Limestone	6	6 05	552	7.0	1,414	1,703	1,558
	Average of acid, calcium free, concentrated fert. 2–7		33	5.37	433	8.6	1,368	1,317	1,343
9	Triple superphosphate, urea, potassium sulfate	None	24	5.35	481	12.5	1,426	1,406	1,416
10	No fertilizer			5.38	89	4.0	433	727	585
	Average 1 to 9, all fertilizer applied at planting					10.4	1,377	1,381	1,379
	Average 1 to 9, one-half fertilizer applied at planting, one-half as side dressing					7.3	1,382	1,394	1,388

* Each fertilizer was used in two ways, all before planting, and one-half before planting and one-half as a side-dressing. The average for the two methods of application is given at the bottom of the table.

† Fertilizer on Plot No. 1, analysis 6–8–4, application 600 pounds per acre.

** Fertilizer on Plots No. 2–9, analysis 12–16–8, application 300 pounds per acre.

†† Nitrogen supplements furnished, one eighth of nitrogen in fertilizer. Limestone (Plot 8) 600 pounds per ton of 12–16–8.

15

In the last three years of the experiment, substantial and interesting yield differences were obtained that apparently can be attributed to specific properties of the fertilizer. The 6–8–4 fertilizer produced 165 pounds more seed cotton than the average of the more acidic concentrated fertilizers used in plots 2–7. The use of supplements of standard inorganic or organic sources of nitrogen had little or no influence on yields obtained with concentrated fertilizers. The outstanding result was the increase in yield when the concentrated fertilizer was supplemented with limestone to make the fertilizer slightly basic. That fertilizer, plot 8, produced 437 pounds more than the corresponding concentrated fertilizer, plot 5, and 221 pounds more than the standard 6–8–4 fertilizer.

The results of this experiment are similar to those noted on some fields in series 1 and 2: during the first years of the experiment, the concentrated fertilizers were as effective as the standard fertilizer, but their efficiency decreased with time. This experiment indicates that the decrease in efficiency of the concentrated fertilizers on continued use is not prevented by use of supplements of standard organic or inorganic sources of nitrogen. A supplement of limestone, on the other hand, increased the efficiency of the concentrated fertilizer and made it more efficient than the standard 6 -8–4, which was slightly acidic.

In this experiment, there was considerable variation in the source of nitrogen in the various fertilizers. On plots 2, 3, and 4, considerable nitrate nitrogen was used; two plots in addition to the 6–8–4 had organic nitrogen in the form of cottonseed meal and tankage. None of these variations in source of nitrogen appeared to influence yield materially. The nitrogen, in the highest yielding fertilizer, made basic by limestone, was derived from ammonia salts and urea.

SERIES 4: THE INFLUENCE OF SUPPLEMENTS OF MINOR ELEMENTS AND LIMESTONE ON THE EFFICIENCY OF CONCENTRATED FERTILIZERS

Experiments were started in 1930 to determine whether or not application of minor elements, such as copper, manganese, nickel, zinc, and boron, influenced the efficiency of concentrated fertilizers. An experiment at one location included limestone as a supplementary treatment and at another, magnesium sulfate as a supplement with and without limestone. The field at Goldsboro, N. C., was adjacent to that just discussed in the preceding section. The other was on Cecil clay at the Georgia Agricultural Experiment Station near Griffin, Ga.

The results on Norfolk sandy loam at Goldsboro, N. C., are given in table 9. The treatments were repeated four times. As in preceding tables, yields are given for the first and second parts of the experimental period. The first three fertilizers were acid, and also calcium free, whereas fertilizers 4 to 6 were slightly basic and contained a large quantity of calcium. The limestone may also have contained small quantities of magnesium.

The addition of minor elements to unlimed and limed soil was not beneficial during the first three years of the experiment and reduced yields during the last two years.   The highest yields were secured when limestone was used as a supplement.   During the first three years, the use of limestone alone as a supplement increased the yield 151 pounds per acre, as shown by the results from fertilizers 1 and 4.   In the last two years, the increase due to limestone averaged 513 pounds of seed cotton per acre.   In all cases, the use of a limestone supplement materially increased yields secured from the use of the concentrated fertilizers.

The details of fertilizer treatments and yields of seed cotton on Cecil clay at Griffin, Ga., are given in table 10.   The treatments were repeated four

TABLE 9

Influence of minor elements and limestone supplements on yield of seed cotton obtained with concentrated fertilizers on Norfolk sandy loam

FERT. NO.	MATERIALS ADDED* TO FERTILIZER (12–16–8; 450 LBS PER ACRE, MADE WITH AMMONIUM PHOSPHATE, UREA, AND POTASSIUM SULFATE)	REACTION OF SOIL 1934.	AV YIELD OF SEED COTTON PER ACRE		
			1930–1932	1933–1934	1930–1934
		pH	*lbs.*	*lbs.*	*lbs.*
1	None	5.0	1,519	1,375	1,461
2	Manganese sulfate	5.1	1,588	1,291	1,469
3	Manganese, copper, nickel and zinc sulfate, borax	4.9	1,493	1,225	1,385
4	Limestone	6 3	1,670	1,888	1,757
5	Limestone, manganese sulfate	6.2	1,691	1,632	1,667
6	Limestone, manganese, copper, nickel and zinc sulfate, borax	6.3	1,653	1,699	1,672
	Average of acid, calcium-free concentrated fertilizers	5 0	1,533	1,297	1,438
	Average of concentrated fertilizers with limestone supplement	6.3	1,671	1,740	1,699

* Manganese sulfate was applied at the rate of 50 pounds per acre; copper, nickel, and zinc sulfate at the rate of 5 pounds; borax 2 pounds and limestone, 600 pounds per acre.

times.   During the first, as well as the latter, years of the experiment, the acid, calcium-free and magnesium-free concentrated fertilizers used on plots 2, 3, and 4 produced decidedly lower yields than the standard 5–9–3 fertilizer. Supplementing the concentrated, acid fertilizers with minor elements did not affect yields materially.   When used with limestone, the minor elements apparently had a slight beneficial effect.

The outstanding result of this experiment was the response from supplements of magnesium sulfate and limestone, when used alone or together. Considering the average yield for the entire period, magnesium sulfate increased the yield from 494 to 695 pounds and calcium limestone increased it from 494 to 707 pounds.   Neither treatment, however, resulted in as good yields as were obtained with 5–9–3: 778 pounds.   When the magnesium

sulfate and calcium limestone were used together as supplements, a yield of 875 pounds resulted. This is 97 pounds more than was obtained with the standard 5–9–3. It appears from this experiment that the poor results from use of some concentrated fertilizers on certain acid soils are due to the acidity of the fertilizer and to a deficiency of calcium and magnesium. There is little evidence to indicate that the minor elements, copper, nickel, zinc, and boron, are important factors in these soils. Nor have experimental data been reported in literature showing a need of minor elements for cotton on soils of any

TABLE 10

The influence of minor elements and limestone supplements on yield of seed cotton obtained with concentrated fertilizers on Cecil clay at Griffin, Ga.

PLOT NO.	FERTILIZERS (EQUIV. TO 900 LBS. OF 5–9–3 PER ACRE)	MATERIALS* ADDED TO FERTILIZER	REACTION OF SOIL 1935	AV. YIELD OF SEED COTTON PER ACRE		
				1930–1932	1933–1935	1930–1935
			pH	*lbs.*	*lbs.*	*lbs.*
1	5– 9–3†	None	4.9	807	739	778
2	15–27–9**	None	4.6	562	427	494
3	15–27–9	Manganese	4.6	589	415	502
4	15–27–9	Manganese, zinc, nickel, copper, boron	4.8	579	380	480
5	15–27–9	Magnesium	5.0	747	642	695
6	15–27–9	Limestone	5.9	763	658	707
7	15–27–9	Limestone, manganese	5.6	818	723	770
8	15–27–9	Limestone, manganese, zinc, copper, nickel, boron	5.8	878	731	805
9	15–27–9	Limestone, magnesium	6.1	945	804	875

* Manganese sulfate was applied at the rate of 50 pounds per acre; copper, nickel, and zinc sulfate at the rate of 5 pounds; borax, 2 pounds; magnesium sulfate, 50 pounds; and limestone, 600 pounds per acre.

† Fertilizer contains sodium nitrate, ammonium sulfate, cottonseed meal, superphosphate, and potassium sulfate.

** Fertilizer contains ammonium phosphate, urea, and potassium nitrate.

extensive area; manganese, zinc, and boron deficiency have been noted on other crops in soils of the cotton belt. Such soils may be improved for cotton by their use.

SERIES 5: INFLUENCE OF NEUTRALIZING STANDARD AND DOUBLE STRENGTH FERTILIZERS

Most of the data of the preceding experiments were obtained by 1933 and indicated the importance of regulating the equivalent acidity of concentrated fertilizers, as well as their content of the secondary elements, calcium and magnesium. Experiments were started in the spring of 1934 to ascertain the effect, when used over a period of years, of acid-forming and neutral fertilizers on cotton soils of different reaction and physical characteristics. Six soils in North Carolina and one in Virginia were included. A second group of

experiments in this series was inaugurated on five soils in North Carolina, South Carolina, and Georgia in 1936. The experiments of the first group are detailed in table 11. Each treatment was repeated four times.

The data of three years on the Norfolk (expt. 1) and Cecil soils (expt. 2) are averaged, as the trend of results was very similar each year. Likewise, the data of the Ruston soil (expt. 3) are averaged for two years, but the crop failed the third year because of drought. The data on the Dunbar (expt. 4), Greenville (expt. 5), Marlboro (expt. 6), and Onslow (expt. 7) soils are averaged for the first two years and given separately for the third year, as there were no significant differences in yields from acid-forming and neutral fertilizers in the first period. In this period, the yields from acid-forming double strength fertilizers were as large or larger than those from the acid-forming standard fertilizers. The relative effects were different when these experiments were continued the third year.

In experiment no. 1, the standard and double-strength acid fertilizers gave approximately the same yields, and these were more effective when lime was included. The largest yield resulted from the neutral double-strength fertilizer. The soil had a pH of about 5.0. The acid-forming fertilizer reduced the pH of the soil slightly. In experiment no. 2, the acid concentrated fertilizers were not as effective as the standard fertilizer; however, the neutral concentrated mixtures gave results comparing favorably with the standard fertilizer. The acid and neutral fertilizer influenced the pH of the soil, which was originally about pH 5.5. In experiment no. 3 (pH 5.6–5.7), the standard fertilizer gave larger yields than the double-strength fertilizer; however, the addition of lime to the standard fertilizer did not make it more effective. The acid double-strength fertilizers were improved by including lime. Yields from the two grades of neutral fertilizer are somewhat similar.

The acid concentrated fertilizer gave approximately the same, or larger, yields than the acid standard fertilizer the first two years in experiments nos. 4, 5, 6, and 7. Within this period, there were no significant differences in yield resulting from the inclusion of lime in the two grades of fertilizers. These soils had a pH of 5.4, 7.5, 6.0, and 6.2, respectively. In the third year, the neutral fertilizers were relatively more effective than acid-forming fertilizers. The yield from the standard fertilizer was as large or larger than that from the concentrated, although during the first period the latter led to larger yields than the standard fertilizer. In experiment no. 6, the acid standard fertilizer gave a larger yield than the acid, calcium-free, concentrated fertilizer, and as large or larger yield than neutral standard fertilizer. The results on this soil indicate that the relatively poor returns from the concentrated fertilizer may be due to lack of calcium. No results were obtained from the double-strength fertilizer in 1936 in experiment soil no. 7. The yields from neutral standard fertilizers were larger this year than those from acid fertilizers. The acid-forming fertilizers had reduced the pH of the soil appreciably in the third year, while the pH of the neutral fertilizer plots remained about the same.

TABLE 11

Results of experiments in first group of series 5: Effect of limestone in acid fertilizers on cotton yields (per acre) on seven soils in North Carolina and Virginia

NO.	FERTILIZER ANALYSIS N-P2O5-K2O	SUPPLEMENT ADDED TO FERTILIZER	EQUIV. ACIDITY PER UNIT N	EXPT. 1 NORFOLK SANDY LOAM GOLDSBORO, N.C. pH 5.0-5.1 Av. 1934-1936	EXPT. 2 CECIL CLAY LOAM YOUNGSVILLE, N.C. pH 5.5 Av. 1934-1936	EXPT. 3 RUSTON SANDY LOAM FAYETTEVILLE, N.C. pH 5.5-5.7 Av. 1934-1935	EXPT. 4 DUNBAR SANDY LOAM WINDSOR, N.C. pH 5.4 Av. 1934-1935	EXPT. 4 1936	EXPT. 5 GREENVILLE SANDY LOAM GARYSBURG, N.C. pH 7.5 Av. 1934-1935	EXPT. 5 1936	EXPT. 6 MARLBORO SANDY LOAM ROWLAND, N.C. pH 5.9-6.0 Av. 1934-1935	EXPT. 6 1936	EXPT. 7 ONSLOW SANDY LOAM HOLLAND, VA. pH 6.0-6.2 Av. 1934-1935	EXPT. 7 1936
				lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
1	4-8-4*	None	91	1,288	1,137	1,536	1,430	1,300	1,230	1,080	2,125	1,605	1,195	1,023
2	4-8-4	Calcium limestone	0	1,353	1,237	1,510	1,398	1,337	1,225	1,206	2,275	1,587	1,072	1,370
3	4-8-4	Calcium limestone, magnesium limestone	0	1,333	1,204	1,500	1,341	1,375	1,218	1,120	2,087	1,630	1,116	1,310
4	4-8-4	Dolomitic limestone	0	1,325	1,078	1,575	1,420	1,475	1,262	1,323	2,050	1,430	1,089	1,230
5	4-8-4	Same as 4, except urea replaces cottonseed meal	0	1,318	1,050	1,615	1,345	1,412	1,144	1,120	2,125	1,350	1,129	1,290
6	8-16-8†	None	89	1,267	989	1,375	1,552	1,312	1,356	1,012	2,150	1,450	1,179	......
7	8-16-8†	Dolomitic limestone	0	1,426	1,138	1,496	1,406	1,412	1,407	1,404	2,025	1,675	1,295	......
8	8-16-8†	None	60	1,266	947	1,432	1,541	1,400	1,586	1,204	2,062	1,800	1,179	......
9	8-16-8**	Dolomitic limestone	0	1,337	1,265	1,583	1,581	1,588	1,587	1,436	2,275	1,740	1,056	......
X	No fertilizer			638	725	826	812	688	568	459	1,375	1,025	629	620

* 4-8-4 fertilizer contains ammonium sulfate, cottonseed meal, superphosphate, and potassium sulfate, except for changes and supplements indicated.

† Contains ammonium phosphate, urea (50 per cent of N), ammonium sulfate.

** Contains ammoniated triple superphosphate, urea (50 per cent of N), ammonium sulfate, potassium nitrate, and potassium chloride.

In the second group of experiments in this series inaugurated in 1936 on five soils in North Carolina, South Carolina, and Georgia, an acid 6–8–4 fertilizer containing ammonia salts was compared with a fertilizer of the same composition, neutralized with dolomitic limestone. Another comparison was made with each fertilizer by withholding half of the nitrogen for application as a side dressing. In tests replicated five times on each soil, the yields of cotton were greater in each case from the neutral fertilizer than from the acid fertilizer, giving an average increase of 56 pounds. Both the acid and neutral 6–8–4 applied at planting generally gave significantly larger yields than a 3–8–4 side dressed.

The results of this series of experiments as a whole show the advantage of neutral over acid fertilizers and the advantage of supplementing concentrated fertilizer with limestone.

SUMMARY

The experiments reported were planned to study the effect of concentrated fertilizers and means of making them more efficient on cotton soils of the southeastern states. In the investigations, which covered a period of 12 years, the earlier experiments showed that concentrated fertilizers on some soils were not as efficient as standard strength fertilizers. It was thought that the difference in efficiency might be due to the source of nitrogen; equivalent acidity of fertilizers; content of secondary elements, calcium, magnesium, and sulphur; or content of minor plant food elements, manganese, zinc, copper, and boron.

In experiments on Cecil clay loam at Youngsville, N. C., Cecil sandy clay at Raleigh, N. C., and Norfolk sandy loam at Goldsboro, N. C., concentrated fertilizers, which are acid and calcium-and magnesium- free, gave as good results as standard fertilizer in the early years of the experiment but decreased in efficiency on continued use. On fertile soil at Holland, Va., such fertilizers gave good results during the entire 7-year period of the experiment. Concentrated fertilizers properly supplemented with limestone and, in some cases, with magnesium were usually equal, and frequently superior, to standard fertilizers.

Although it was thought that a deficiency of minor elements in the concentrated fertilizers used in the earlier experiments may have been a contributing cause of their lesser efficiency, the results as a whole indicate that differences in content of minor elements are unimportant in their influence on the relative efficiency of standard and concentrated fertilizers. The sources of nitrogen did not vary widely in their effect on cotton when the fertilizers were properly supplemented with limestone. The largest yields were generally obtained with concentrated fertilizers containing acid-forming ammonia salts or soluble nitrogen supplemented with limestone. This result is in keeping with results of previous experiments cited by Schreiner and Skinner (7), which show that inorganic sources of nitrogen properly formulated may be as effective for cotton

production as higher priced organic nitrogen. Their use thus effectively reduces the cost of fertilizers to the consumer.

The results of these experiments seem of economic importance. They indicate that properly formulated double-strength fertilizers may be as efficient as single-strength fertilizers. As pointed out by Ross and Mehring, (5, 6), double-strength fertilizers, per unit of plant food, cost approximately 20 per cent less than single-strength fertilizers, although both contain the essential plant food elements, including calcium and magnesium. It has also been pointed out that the substitution of soluble for insoluble nitrogen in fertilizers would result in an 8 per cent reduction in the cost of plant food in cotton fertilizers.

The data indicate that the use of properly supplemented concentrated fertilizers, most economically applied simultaneously with planting and most advantageously applied in bands to the side of the seed, should give good results and reduce the cost of cotton production.

REFERENCES

(1) CUMINGS, G. A., MEHRING, A. L., SERVISS, G. H., AND SACHS, W. H.   1931   Progress report on mechanical application of fertilizers to cotton in South Carolina—1930.   *U. S. Dept. Agr. Cir.* 192: 29.

(2) CUMINGS, G. A., MEHRING, A. L., SKINNER, J. J., AND SACHS, W. H.   1933   Mechanical application of fertilizers to cotton in South Carolina—1931.   *U. S. Dept. Agr. Cir.* 264; 33.

(3) GADDUM, L. W., AND ROGERS, L. H.   1936   A study of some trace elements in fertilizer materials.   Florida Agr. Exp. Sta. Bul. 290.

(4) MANN, H. B.   1933   Machine application of fertilizers to cotton.   Discussion of the North Carolina experiment.   *Proc. ninth Ann. Meeting Joint Com. Fert. Application:* 38–46 (mimeograph).

(5) ROSS, W. H., AND MEHRING, A. L.   1936   Influence of concentration as a factor in the retail price of fertilizer.   *Indus. and Engin. Chem.* 12: 430–438.

(6) ROSS, W. H., AND MEHRING, A. L.   1936   Methods for reducing the retail cost of cotton fertilizers.   Commercial Fert. Yearbook—1935.

(7) SCHREINER, O., AND SKINNER, J. J.   1934   Adaptation of fertilizers for cotton soils.   *Amer. Fert.* 81: 5–7.

(8) SKINNER, J. J.   1932   Machine application of fertilizers on cotton in South Carolina—1932.   *Proc. eighth Ann. Meeting Joint Com. Fert. Application:* 48–50.

(9) SKINNER, J. J., AND ADAMS, J. E.   1933   Machine application of fertilizers to cotton.   Discussion of the South Carolina experiments.   *Proc. Ninth Ann. Meeting Joint Com. Fert. Application:* 37–38.

PHYSICAL PROPERTIES OF SOILS THAT AFFECT PLANT NUTRITION[1]

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Conditions determining the accessibility of moisture and nutrients are important in the nutrition of plants. Roots must be able to penetrate the soil and to develop the absorbing system of growing points and root hairs. Absorption of nutrients and moisture is dependent upon many contacts between the roots and the soil.

Three physical properties of the soil, texture, structure, and depth, principally determine the development and functioning of the root system. The same soil properties control moisture capacity and usable water, drainage and aeration, and the permeability and penetrability.

The orchards with which this study deals have been under observation for a number of years. Tree growths, yields, moisture records, and now the soils, have been given attention. This report is based on the study of some 30 soils, mostly in walnut or filbert orchards. The data presented are typical of those obtained throughout the work.

METHODS OF STUDY

The moisture relationships of the soils were first considered. Field moisture capacity (11) was obtained by determination of the amount of moisture held after rains had satisfied the capillary capacity of the soil, and the gravity water had drained away. For this purpose, soil samples were obtained 2 or 3 days after rains had saturated the soil with water.

Wilting percentages were determined by the sunflower method (11). Soil samples taken to a depth of 6 feet at 1-foot intervals were used for this work. There was thus obtained a wilting percentage for each foot level for the various soils. Available moisture was obtained by subtracting wilting percentage from field capacity percentage.

The Bouyoucos method (2) was employed for the mechanical analyses of the soils.   This method depends upon so adjusting the time of sedimentation that the soil particles of different sizes may be estimated with the hydrometer.

A method for the study of the natural soil aeration, by which chunks of natural soil as large as a 5-inch cube could be obtained, was devised by "trial and error."   An 8-inch hole was bored 10 feet deep with a rotary post hole digger, and chunks of soil were taken from one side of the hole with a two-lipped plunger type of post hole digger.   This latter digger had jaws made of $\frac{1}{4}$-inch circular saw steel and interchangeable iron pipe handles of varying lengths. Samples were taken at 6-inch intervals in the surface foot of soil and below that at 1-foot intervals.   The samples were preserved in tight cans.

The digger was set with the outer jaw about 4 inches back from the edge of the 8-inch hole and then driven down with a sledge hammer.   When driven to the requisite depth, a full sized core or chunk could be broken off and taken out practically undisturbed.   In order to break out the core, it was necessary to dig a narrow trench on the opposite side of this hole so that the handles could be bent over far enough to release the sample.   With this equipment, satisfactory samples could be taken to a depth of 10 feet, except where rock, gravel, or sand interfered.

A good sized chunk of moist soil was whittled down to the shape of a potato (preferably about 200 gm. weight) without disturbing the natural structure. Care was used to eliminate all soil that had been compressed or disturbed in any way by the process of sampling.   The prepared soil was weighed, and the volume determined by displacement of light oil in a graduated cylinder.   Several replications of these determinations were made.   The replicated samples checked closely.   Moisture was determined at the same time on a sample of the shavings from the same soil.   From these data, apparent specific gravity, or volume weight, was calculated.

The rim method was used in sampling a few sandy soils.   A metal rim of measured volume was pressed into the soil in the side of a trench and sliced level with the surface to obtain a sample.   This was weighed, and the data were utilized for apparent specific gravity and porosity estimations.

Real specific gravity was obtained by the usual pycnometer method (5). From the real and apparent specific gravities, which are not reported for lack of space, porosity was calculated.   Volume occupied by water at the time of sampling was calculated from the moisture percentage at that time.   Likewise, volume occupied by water at field capacity was calculated from the percentage of moisture held by the soil in the field after gravity water has drained away.   Space occupied by air is the difference between total porosity by volume and the volume occupied by water.[2]

The division into capillary and non-capillary porosity is based upon the

[2] This method of obtaining air space at field capacity is objectionable if the soil is at low moisture content at the time of sampling.   Clay of the heavy soils shrinks from drying. Sampling in the spring, when the soil is near field moisture capacity, is desirable.

usual classification of moisture into hygroscopic, capillary, and gravity water. The capillary water occupies capillary spaces.   Gravity water passes into and through non-capillary spaces into the drainage.   When the gravity water disappears, the non-capillary spaces are left open for soil aeration.   Thus, non-capillary and air space are synonymous and represent percentage of total soil volume in the form of voids unoccupied by water at field moisture capacity.

THE PROPERTIES OF HEAVY SOILS

Clay more or less controls the air-moisture-root relationships in the soil· The clay or colloid is so fine that openings between the particles are extremely small or, sometimes, negligible.   Jelly-like colloids moistened with imbibitional water may have no openings through which air may enter, because of swelling which tends to close all pore-spaces.   Such soils are either water-saturated or contain only dead air spaces most of the time.

TABLE 1

Physical properties and moisture relationships of Sites clay

SOIL HORIZON	FINE SILT AND CLAY	COLLOID	PORE SPACE	WATER AT FIELD CAPACITY (CAPILLARY SPACE)	AIR SPACE (NON-CAPILLARY)	USABLE WATER	USED WATER
	0.0102–0.0 mm.	0 002–0 0 mm.	Per cent of soil volume	Per cent by volume	Per cent by volume	Per cent by volume	Per cent by weight
depth in feet	per cent	per cent					
0–0.5	64.6	24 4	52 3	46 4	5.9	15	13
0.5–1	68.3	29.4	55 8	42.8	13 0	15	13
1–2	71.3	33.4	56.9	43 0	13 9	13	9
2–3	70 3	35 4	57 4	48.3	9 1	13	7
3–4	82 3	38.9	58 1	52 3	5 8	12	5
4–5	70 3	38 5	57.8	57.9	−0.1	18	6
5–6	70.3	39.5	62 3	64 7	−2.4	7	4
6–7	64 6	35.5	59.1	. .	.	..	..
7–8	56 6	25 5	58.8	....	....	..	..

Because of these properties of clay, circulation of air to any great depth in heavy soils is limited.   Other characteristics are a high moisture capacity and, sometimes, a nearly equally high wilting point, with a relatively low percentage of usable water.   These points are brought out in the data of tables 1 and 6, which show some physical properties of a heavy Sites clay to a depth of 8 feet.

The data of table 1 indicate that only about half the usable water (difference between wilting point and field capacity) from the third foot to the sixth foot was used during the season, though Persian walnut trees growing on this soil suffered from lack of water.   Distinction between supposedly usable water and "used" water becomes necessary.

Vageler (9) reports that certain heavy tropical clays are made up of so much

fine material that the field moisture capacity is 60 to 70 per cent, and the wilting percentage nearly as high. Such soils must be wet to a paste before moisture can be given up to plants. There is, therefore, practically no available water at any moisture content, and the soils are unsuited to agriculture.

The above observations indicate that only moisture held in the larger spaces between granules in heavy soils is usable by plants. Though the granules of colloids are porous like a sponge, the water held within these pores would not be available. In the absence of granulation, such heavy soils could supply little moisture for plant nutrition.

The moisture equivalent and field moisture capacity are nearly equal in many soils. Thomas (7) gives $10\,\mu$ as the largest diameter of pores holding moisture against a force 1,000 times gravity. This is assuming that pore space diameter is equal to soil particle diameter. If these figures are accepted as representing field conditions, available soil moisture is that portion of the total moisture held in the capillary spaces of a size range of $10\,\mu$ diameter and smaller. This order of magnitude permits movement of moisture from the soil into the absorbing root system.

The data of table 1 indicate that heavy soils may be water-logged, in the sense that all the pore space is filled with water, at field capacity. A free water table was not found in this Sites clay, except in the rainy season, and yet, in July, at a depth of 5 and 6 feet, no pore space was unoccupied by moisture. This is a case of saturated soil without a water table, a more or less permanent condition. Only a few of the larger capillaries slowly give up their moisture from the tight horizons.

Root studies to be reported elsewhere indicate that few roots penetrate lower than 5 feet in this soil. Most of the roots are in the top 3 or 4 feet of soil. The Persian walnut trees on this area are compelled to survive and produce a crop principally from moisture in the root zone, as is indicated by the large amount of moisture remaining in the deep soil below the roots at the end of the season.

According to physical laws, small capillaries cannot give up moisture to larger capillaries. Therefore, there is no possibility of moisture passing upward from a tight subsoil to the more open and permeable soil above. Under such conditions, plants may wilt and die because of a lack of moisture in the upper soil even though there is an abundance of water immediately below. The moisture is inaccessible and unavailable.

The larger capillary and the non-capillary porosity have received too little consideration. One per cent of pores of the size afforded by sand and coarse silt may allow many times as rapid water penetration and movement as is possible if all pores are of the size afforded by fine silt and colloidal particles. Not how great is the porosity but how large are the openings is the more important condition in plant-moisture relationships. Soils having the largest percentage of pore space may have the smallest pores and be least suited to support plant growth.

In this work, no assumption has been made as to whether pore space is an assemblage of small cellular units or of irregular, thread-like tubes. Neither has it been assumed that water is present as a film, or that it is an indistinguishable part of a colloid gel. The nature of the porosity is probably different in different soils. Root channels and worm holes must be continuous openings as far as they extend. Whatever their form, non-capillary pores supply the principal means for water penetration, drainage, aeration, and root growth. Buehrer (4), quoting Russian and German workers, states that the ratio between non-capillary and capillary pore space is the best measure of soil structure yet devised. These Russian workers stress the importance of large size pores in the soil. According to their conclusions, pores smaller than are afforded by particles half a millimeter in diameter are too small for adequate aeration. On this basis, particles or aggregates of medium sand size and larger are necessary to assure good soil structure.

TABLE 2

Shrinkage of Meyer clay adobe and its eff upon soil aeration

SOIL DEPTH*	LINEAR SHRINKAGE ON FIELD SAMPLE	CALCULATED AREA SHRINKAGE	SHRINKAGE ABOVE WILTING POINT (CALCULATED)	AERATION DUE TO SHRINKAGE (CALCULATED)	NON-CAPILLARY PORE VOLUME (NATURAL)	AERATION DUE TO SHRINKAGE, FROM LOSS OF 75 PER CENT OF USABLE WATER (CALCULATED)
inches	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
0–6	11.8	22.2	80.5	17.9	3.5	12.9
6–12	10.4	19.7	78.3	15.4	3.5	11.6
12–18	8.7	16.6	74.0	12.3	10.4	9.2
18–24	7 2	13.9	94.4	13.1	5.1	10.8
24–30	7.9	15.2	85.0	12 9	4.2	9.7
30–36	7.3	14.1	74.0	10.4	6.6	7.8

* Field moisture capacity is 34.5 per cent, 32.2 per cent, 30.3 per cent in first, second, and third feet respectively. Wilting point is 17.2 per cent, 17.6 per cent, 17.0 per cent, respectively, for same depths according to Work and Lewis (11).

SHRINKAGE AND CRACKING, AND SOIL STRUCTURE

The development of structure or the aggregation of fine clay into larger units, therefore, becomes the chief means of overcoming unfavorable effects of heavy textures. Aggregated clay units that function like sand grains tend to give the properties of a lighter soil. Only natural processes can develop soil structure. The alternate wetting and drying of the soil causes periodic swelling and shrinking. These changes develop granulation and structure. The entire surface soil may gradually break into small irregular chunks and granules, due to alternate wetting and drying, which set up stresses that shatter the hardest clods. The shrinkage opens non-capillary spaces that serve a useful purpose. The data of table 2 indicate the extent to which shrinkage may aid in opening a tight soil.

The non-capillary space which contains air instead of water in this soil is only 4 to 6 per cent of the total, when the soil is at its field moisture capacity. By contrast, shrinkage from drying opens cracks to the extent of 14 to 20 per cent of the surface area. The last column of table 3 indicates air space opened by cracks, when 75 per cent of the usable water has been removed. Cracking, therefore, becomes an effective means of aerating the heavy soil as plants use moisture and the soil approaches the wilting percentage. This means of aeration may be effective in the surface soil but is decreasingly effective with increase in depth. Aeration from shrinkage and cracking, while helpful, is not adequate for best plant growth.

All soils containing any considerable amount of clay crack more or less upon drying and thus improve the aeration and offer opportunities for root penetration. The interfaces of cracks are sometimes covered in the deep soil with

TABLE 3

Shrinkage and change in density in relation to aeration of Meyer clay adobe

SOIL DEPTH	REAL SPECIFIC GRAVITY	APPARENT SPECIFIC GRAVITY WET*	APPARENT SPECIFIC GRAVITY DRY	PORE SPACE BY VOLUME* WET	PORE SPACE BY VOLUME DRY	MEASURED SHRINKAGE BY VOLUME	CALCU-LATED SHRINKAGE VOLUME	INCREASED DENSITY
inches				*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
0–6	2 72	1.34	1.88	50.8	30.9	28.7	31.2	40.3
6–12	2.73	1.34	1.90	50 9	30.4	29.5	28.0	41.8
12–18	2.73	1.38	1.91	49.9	30.0	27.2	23.8	38.4
18–24	2 74	1.51	1.97	44.8	28.1	23.3	20 0	30 5
24–30	2.74	1.49	1.94	45 6	29.2	23 3	21 8	30.2
30–36	2.74	1.44	1.94	47 4	29.2	25.8	20.3	34.7
Average.............		1.42	1.92	48 2	29.6	26 3	24 2	35.98

* Calculated to water free basis. This soil contained approximately 63 per cent clay, varying from 62.6 to 63.6 per cent in the different horizons. The colloid content was 54 per cent.

mats of fine absorbing roots. Excessive cracking may prove harmful, but it is perhaps less so than if there were no cracks and there were a consequent lack of means of providing air for oxidation processes and root respiration. Vageler (9) says that cracking is helpful in aerating heavy tropical soils. The cracks also provide easy penetration of water until swelling seals the surface of the soil by closing the cracks.

The pronounced effect of drying and shrinkage on soil properties is indicated in the data of table 3. The apparent specific gravity and the pore space were determined on samples of fresh, moist soil (at about field moisture capacity) and on the water free soil. Shrinkage was measured both volumetrically (after slow drying in a moist chamber and then in the oven) and by calculation from linear shrinkage on field samples with the natural structure undisturbed. The following formulae were used for shrinkage calculations:

$L = $ Linear shrinkage in per cent

$A = 2L - \dfrac{L^2}{10^2}$ —area shrinkage in per cent

$V = 3L - \dfrac{3L^2}{10^2} + \dfrac{L^3}{10^4}$ —volume shrinkage in per cent

The calculated volume shrinkage agrees with the measured shrinkage in a general way, in that a large shrinkage is indicated in both instances. The increase in density from 30 to 40 per cent indicates that as the soil slowly dries, the pore spaces collapse and afford little aeration to the plant roots within these spaces.

The data indicate that the principal aeration of the dry soil is through the cracks which open as the soil mellows and granulates. The soil shrinks in three dimensions in developing granulation, but gravity causes the cracks in a horizontal plane to close or, more probably, prevents their formation. Work at the Medford, Oregon, U. S. Pear Investigation Laboratory (private communication) shows that the surface of the soil rises and falls with change in moisture content. After irrigation, the surface soil swells, and the whole topography rises. As the moisture is removed from the soil, the surface elevation recedes again. This change in elevation is great enough to be measured.

Silt does not shrink and crack and, consequently, does not develop any pronounced granulation. In the absence of shrinkage and swelling, such things as organic matter, roots, insects, and freezing and thawing are important in developing structure. Though silt particles are much larger than clay, soils very high in fine silt are sometimes more refractory than the more clayey soils. Silt grains are too small for good porosity and structure and, lacking clay, there is insufficient binding material for developing larger aggregated units. Hence the lack of structure in silt that is low in clay. Thus, shrinkage and swelling, and resulting granulation are saving features of heavy clay soils, and a certain percentage of clay in all heavy soils is favorable for granulation and structural development.

DOWNWARD MOVEMENT OF COLLOID

Heavy soils may have surface horizons showing good structural development to variable depths. This has been found true of all the soils studied to date. In some of the older formations, the fine colloid has gradually filtered downward until the deeper horizons are so filled with fine material that the percentage of small capillary pores is large, and the non-capillary pores are negligible or lacking. Bray (3) states that the downward movement of clay particles is freest when they are 50 millimicrons in diameter or less. The natural tendency is for the clay to filter into the larger pores of the soil. The fine clay is carried where water movement is freest. In some cases, clay

appears to have completely filled former open channels as large as 8 mm. in diameter. Some channels are only partially filled, whereas others remain entirely open. A sample of clay picked from the channel which it had filled had a moisture content of 53 per cent at the time of sampling. The surrounding soil averaged 36 per cent moisture at the same time.

The data in table 4 indicate that there is a downward movement of colloidal clay into the deeper horizons. In the old soil formations, the colloid content usually increases to a certain depth and then diminishes. In the recent formations, there is a smaller quantity of colloid, and its distribution is more uniform throughout the profile, as is indicated by the Chehalis series. The Willamette series shows a similar nearly uniform distribution of colloid. Both these soils are deep and well drained and suited to deep-rooted crops. The hill formations, such as Melbourne, Aiken, and Sites, are those of limited root penetration and low available moisture in the deep horizons. Some of the Melbourne

TABLE 4

Fine clay (2μ–0.0μ) in different horizons

SOIL SERIES

depth in feet	Melbourne I	Melbourne II	Aiken	Sites	Chehalis	Willamette
	per cent	per cent	per cent	per cent	per cent	per cent
0–0.5	18.8	12.0	23.4	24.4	10.2	15.6
0.5–1	19.3	18.0	24.0	29.4	10.2	16.0
1–2	22.3	33.6	32.2	33.4	9.2	15.6
2–3	24.8	33.6	36.0	35.4	8 2	15.6
3–4	42.8	40.6	40.0	38.9	9.2	17.0
4–5	42.9	12.6	....	38.4	9.2	16.0
5–6	36.8	12.6	....	39.5	8.8	18.0
6–7	29.8	....	....	35.5	....	17.4
7–8	.. .	....	....	25.5	. ..	14.0

soils change from heavy clay to light sandy material at a depth of 4 or 5 feet and afford better conditions for root penetration. Moisture is used by tree roots in the light horizons of the Melbourne soil but not in the heavy horizons of the Sites soil.

The oldest soils of western Oregon are in the hill formations. The clogging of the circulatory system of such old soils is the natural consequence of age. Loss of lime through leaching makes the soil acid and leads to increased dispersion. It is only a question of time until the infiltrating muddy water closes internal drainage pores with colloid. Clean cultivation in orchards, with lack of soil protection in winter, only hastens the clogging process. Under such conditions, there is more muddy water from the unprotected surface to filter into such openings as are present in the soil.

In soils with a high water table, infiltrating clay concentrates in a thin horizon and close to the surface. There is no means of removing much of the

water that is saturating the tight horizons. Consequently, there is little shrinkage and cracking to develop granulation or to afford aeration, and as a result few roots can develop. The root zone is principally above this tight horizon. Such soils are extremely droughty and unsuited to summer crops without irrigation. The shallow rooted crops may succeed well if irrigated. Irrigated Ladino clover pastures are good on the Dayton series. Some varieties of strawberries do well. Trees and other deep rooted crops are a failure.

ROOTS AND MOISTURE RELATIONS OF HEAVY SOILS

The few roots found in heavy horizons are often tightly held and more or less compressed or squeezed out of shape. Growing root tips are probably from

TABLE 5

Physical properties and moisture relationships of Newberg loam

DEPTH	FINE SILT + CLAY*	PORE SPACE VOLUME	CAPILLARY PORE VOLUME	NON-CAPILLARY PORE VOLUME	FIELD MOISTURE CAPACITY BY WEIGHT†	WILTING PERCENTAGE BY WEIGHT†	USABLE WATER BY WEIGHT†
feet	per cent	per cent	per cent	per cent	per cent	per cent	per cent
0–1	55	55	41	14	34	16	18
1–2	43	57	39	18	34	14	20
2–3	33	57	40	17	37	16	21
3–4	53	57	41	16	38	15	23
4–5	51	58	41	17	37	16	21
5–6	41	58	42	16	37	13	24
6–7	27	56	36	20	..	.	..
7–8	34	57	40	17	..	..	..
8–9	36	57	46	11	.	..	..
9–10	41	57	50	7	..	..	..
10–11	49	58	48	10	.	..	..
11–12	51	58	52	6	..	..	..

* Fine silt + clay = 0.02 mm. and smaller, Bouyoucos method (2).
† Moisture studies have been limited to six feet depth in all soils, because the importance of greater depths was not realized when the study was initiated.

100 to 500 μ in diameter. These growing points find little encouragement for development in soil horizons with particles mostly less than 10 μ in diameter. Tight soils must offer great mechanical resistance to root penetration. This and the lack of air for their respiration no doubt cause most of the roots to stop at the tight horizons.

Deep soil is an effective moisture storage reservoir if the roots are able to utilize the moisture. Hall (6) states that during the dry summer of 1870 a barley crop at Rothamsted removed 900 tons of water an acre from above the 54-inch level. The data were obtained by moisture comparisons on cropped and fallow soils. On this basis of 200 tons of usable, stored soil moisture an acre for each foot depth, 10 feet of storage volume would provide 2,000 tons of water for summer growth in good soils.

The soils most capable of supporting good summer growth are those having favorable texture and good structure to the greatest depth. Soils having lighter texture with increasing depth are favorable, if not too sandy. The smaller capillaries of the heavier surface soil are able to draw water from the larger capillaries of the deep soil, and thus to lift moisture into the root zone. The desirable properties of a good Newberg loam are shown by the data of table 5.

The moisture relationships in the Newberg loam soil were studied to a depth of only 6 feet, but the soil properties, drainage, and aeration do not change much to a depth of 10 or 12 feet. This soil is supporting a fine filbert orchard. Similar soils produce excellent crops of all kinds. Soils of this type sometimes seem to dry from the bottom up. The free movement of the moisture from

TABLE 6

Fine silt and clay and moisture relationships of diverse soils

HORI-ZON	FINE SILT AND CLAY[*]			FIELD MOISTURE CAPACITY			WILTING PERCENTAGE			USABLE WATER		
	Sites	New-berg	Mel-bourne	Sites	New-berg	Mel-bourne	Sites	New-berg	Mel-bourne	Sites	New-berg	Mel-bourne
depth in feet	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
1	65	55	34	35	34	20	22	16	8	13	18	12
2	68	43	40	36	34	26	23	14	10	13	20	16
3	71	33	45	40	37	28	26	16	20	14	21	8
4	70	53	45	42	38	31	30	15	19	12	23	12
5	82	51	48	49	37	32	32	16	17	17	21	15
6	70	41	24	50	37	32	44	13	13	6	24	19
7	70	27	24	..	..	..	..	..	..	..	..	..
8	64	34	.	..	..	..	.	..	..	..	..	..
9	57	36	13	..	..	..	..	.	..	..	..	..
10	56	41	8	..	..	.	.		.	..	..	..

* 0.02 mm. and smaller.

the deep soil results in easy accessibility to plant roots. In porous, permeable soils of this type, roots develop extensively and utilize the moisture and nutrient resources of a large volume of soil. The amount of usable water is 30 to 50 per cent greater for a similar volume of soil than in the best horizons of the Sites soil previously referred to. The difference in used water is usually still greater. Effective depth in this soil is two or three times that of the Sites.

Comparison of the moisture data of different soil series (table 6) with their characteristic physical properties indicates the inadequacy of studies that are limited to the surface horizons. There may be more usable water in the deep horizons than in the surface of the good Newberg and Chehalis, and of some of the Willamette series. The deeper horizons of tight Aiken, Sites, Salkum, and other series of similar properties have less, and sometimes no, usable water.

ROOT DISTRIBUTION IN THE DEEP SOIL

All soils studied to date show good root development in the top 3 feet of soil. The chief differences in tree root distribution appear in the deeper horizons. Root development is correlated closely with the degree of aeration of the soil. Aeration differences are most marked below a depth of 3 to 5 feet.

In table 7 data are given for the relative root distribution in soils classified as good, average, and poor, according to aeration. Soils or horizons with 10 to 20 per cent of pore space unoccupied by water at field moisture capacity are classed as well aerated; those with 6 to 12 per cent of pore space at field capacity are classed as average; and those with not more than 5 or 6 per cent of pore space unoccupied by water are classed as poorly aerated. These data summarize the results of a root distribution study and represent an average of five or six soils in each class. The fine roots of 2 mm. diameter and smaller were separated from a given volume of soil, and the total length of roots was measured. The smallest total root length was given the value 1, and the other values are relative.

TABLE 7

Relative root distribution of walnuts as affected by soil aeration

CHARACTER OF AERATION	DEPTH OF GOOD AERATION	AIR SPACE BY VOLUME	RELATIVE DISTRIBUTION OF ROOTS (SMALLEST TOTAL ROOT LENGTH = 1)			
			0–3 ft.	4–6 ft.	7–8 ft.	9–10 ft.
	feet	*per cent*				
Good.........	10 or more	10–20	118	36	31	20
Average........	5 to 8	6–12	85	43	24	9
Poor.....	3 to 5	5–10	121	29	3	1

The data indicate the good root development of Persian walnut trees in well aerated soils. There was a gradual tapering off of root concentration through the entire 10-foot profile in the good soils. The data for the average aerated soil show fair root development to a depth of 6 or 8 feet, with a sharp diminution below that depth. The poorly aerated soils contained almost no roots below a depth of 6 feet, and in many cases they became sparse at a depth of 4 to 5 feet.

It is also evident that the major development of fine roots is confined to the surface horizons in all soils. These surface roots probably obtain both moisture and nutrients for tree growth. The deep roots obtain principally moisture, as indicated elsewhere. Failure to develop a deep root system results, therefore, in orchard failure, because of an inadequate moisture supply to the trees during the growing season. Only irrigation can correct the condition, and little information is available to indicate how successful such practice might prove on heavy soils of limited depth.

Aldrich and Work (1), in their study of pears grown on heavy soils, state:

"The inverse relation between evaporating power of the air and rate of fruit enlargement of pears on heavy soils, while soil moisture is well above the wilting percentage, suggests that during periods of high transpiration the leaves lose water at a greater rate than water is supplied by the roots. . . . When the available soil moisture has been reduced to 35 or 40 per cent of the available capacity, the moisture supply to the leaves during the periods of relatively high transpiration is limited by the amount of root area."

These studies indicate that irrigation may not entirely overcome limitation of root development by heavy soils and tight horizons, and that the roots may be too limited to supply adequate moisture to the trees for best fruit production.

AVAILABLE NUTRIENTS OF THE SOIL

The importance of the surface horizons in supplying nutrients for growth is indicated by the distribution of humus and nutrients through the soil profile.

TABLE 8

Humus and available potassium, calcium, and phosphorus in the soil profile

WILLAMETTE LOAM	HUMUS	AVAILABLE P	WATER SOLUBLE K	EXCHANGE- ABLE K	EXCHANGE- ABLE Ca	WATER SOLUBLE Ca
depth in feet	*per cent*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
0–0.5	3.63	357	41.3	420 9	5,805.0	33.0
0.5–1	2.69	266	25.2	340 3	5,515.0	17.70
1–2	1.33	266	19.0	267.0	5,466.0	11.24
2–3	0.36	266	7.3	65.7	5,559.0	11.65
3–4	0.28	266	3 0	91 0	4,408 0	10.17
4–5	0.20	266	1 1	21.0	4,634.0	4.95
5–6	0.18	266	0.85	5.95	5,170.0	1.90

Vigorous plant growth on humid soils is usually associated with a good supply of active humus. The relationship of humus distribution and easily soluble plant nutrients is indicated by the data of table 8. Available phosphorus was determined by the Truog method (8). Exchangeable potassium and calcium were replaced with 0.05 normal hydrochloric acid. Potassium was determined as cobaltinitrate and calcium as the oxalate. Humus was determined by the Walkley and Black (10) modification of the Degtjareff method.

The top 6 inches of the profile contains more humus and generally more available nutrients than the deeper horizons. Nutrients are liberated and prepared for plant absorption by biological processes, especially such processes as nitrification and sulfur oxidation. Usually the most organisms and the greatest biological activity are in the surface or cultivated horizon, and as a consequence, there is a decrease of soluble nutrients with increasing depth of soil.

The above data give emphasis to the importance of the surface or humus horizons. The nature and amount of organic matter, the reaction, and the

supply of easily available minerals determine whether the major absorbing roots of the tree are finding a good feeding ground. In this relationship, the physical and the chemical properties of the soil may be about equally significant. Air, moisture, and nutrients must be supplied in the soil and be accessible to the root system of plants.

How much nutrient is obtained from the deep soil by plant roots is more or less uncertain. But since there is relatively little, and in some cases practically no, easily soluble nutrient material below 3 or 4 feet, in all probability absorption of nutrients below this level is much reduced. There seems to be a better chance for plants to take calcium and phosphorus from the deep soil than there is for the absorption of other materials. Sparsity of roots in the deeper horizons may be due in part to a lack of available nutrients which might stimulate root development.

CONCLUSIONS

Capillary water is retained in the soil by pores of capillary size. The gravity water drains away through the larger non-capillary pores. The larger pores, emptied of gravity water, supply air and afford channels for ramification of the root system throughout the soil mass. More root-soil contacts mean greater efficiency in moisture absorption.

How large pore spaces must be to afford adequate aeration and easy penetration of roots cannot be stated. Clay soils with the internal surface of pores highly hydrated probably require considerably larger openings for adequate aeration and root development than do soils such as sands, that are of a lower degree of hydration. Heavy soils doubtless contain many dead air spaces. Spaces between particles much smaller than half a millimeter in diameter are probably inadequate for the most favorable physical condition of the soil.

Usable water becomes "used" water only if accessible to the root system. In heavy soils, much moisture is classed as usable, but a portion of it is never used, because the roots cannot get to the moist soil nor can the moisture move to the roots. These relationships are governed by the character of the porosity. Depth to which soils are aerated determines the growth and distribution of roots, the utilization of soil moisture, and the suitability of the soils for deep rooted crops. The recent soil formations and some of the best valley floor soils have proved to be good orchard soils. Some of the deepest and best aerated hill soils are likewise supporting good orchards.

Natural processes provide granulation and structural development for a certain amount of aeration in the surface horizons of all soils that are capable of supporting vegetative growth. The significant soil differences appear in the deeper horizons and affect the plant through air-moisture-root relationships. A favorable top soil is not sufficient to assure good tree growth without irrigation.

The zone of greatest development of absorbing roots parallels fairly closely the zone of humus accumulation. This same soil zone carries the greatest

amount of soluble and supposedly available nutrients. Trees fail for the most part when the major root development is confined to the zone of available nutrients. These observations lead to the conclusion that the deep roots serve chiefly for drawing necessary moisture from the deep soil. There must be an advantage, however, in those soils that contribute nutrients as well as moisture from the deep horizons.

Experience has proved that the moisture problem becomes acutely serious on the shallow soils. Favorable soil structure must extend to a depth of 8 or 10 feet to permit deep rooting and the use of moisture. Although more than half the total absorbing root system in the best soils is in the top 3 feet, the remainder (somewhat less than half the total) in the 3- to the 10-foot level is the limiting factor in tree growth and production where irrigation cannot be practiced.

REFERENCES

(1) ALDRICH, W. W., AND WORK, R. A.   1934   Evaporating power of the air and top-root ratio in relation to rate of pear fruit enlargement. *Amer. Soc. Hort. Sci. Proc.* 32: 115–123.

(2) BOUYOUCOS, G. J.   1934   A comparison between the pipette method and the hydrometer method for making mechanical analysis of soils. *Soil Sci.* 38: 335–343.

(3) BRAY, R. H.   1934   A chemical study of soil development in the Peorian Loess region of Illinois. *Amer. Soil Survey Assoc. Bul.* 15, p. 58–65.

(4) BUEHRER, J. F.   1932   The movement of gases through the soil as a criterion of soil structure. Ariz. Agr. Exp. Sta. Tech. Bul. 39.

(5) EMERSON, PAUL   1925   Soil Characteristics. p. 24–25. McGraw-Hill Co., N. Y.

(6) HALL, SIR A. D.   1931   The Soil, ed. 4, E. P. Dutton & Co. Inc., N. Y.

(7) THOMAS, M. D.   1926   The moisture equivalent of soils. *Soil Sci.* 21: 411–424.

(8) TRUOG, E.   1930   The determination of readily available phosphorus in soils. *Jour. Amer. Soc. Agron.* 22: 874–882.

(9) VAGELER, P.   1933   An Introduction to Tropical Soils. Macmillan and Co., London.

(10) WALKLEY, A., AND BLACK, I.   1934   An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci.* 37: 29–38.

(11) WORK, R. A., AND LEWIS, M. R.   1934   Moisture equivalent, field capacity, and permanent wilting percentage and their ratios in heavy soils. *Agr. Engin.* 15: 355–362.

THE SIGNIFICANCE OF INORGANIC SPRAY RESIDUE ACCUMULATIONS IN ORCHARD SOILS[1]

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Orchard spraying methods are unavoidably wasteful of spray materials. A small fraction only of the total amount of spray mixtures used adheres to the fruit. The rest, even though a part adheres temporarily to the wood and foliage, eventually reaches the ground and in time becomes incorporated with other components of the soil mass. In this situation there may be elements of danger to the welfare of both orchard and consumer.

The records of one commercial apple orchard show the distribution of more than 3,500 pounds of lead arsenate per acre during the first 25 years of its productive period. This rather moderate rate of application continued for a total of 50 years will add to the surface soil of that orchard arsenic and lead in amounts that will exceed its native content of both sulfur and phosphorus. Unfortunately, however, these and some other elemental components of inorganic sprays, barium, copper, fluorine, mercury, selenium are neither fertilizing in effect nor entirely inert. There is abundant evidence that under certain conditions each is severely toxic to plant life and when contained in food or feedstuffs becomes potentially harmful to man and beast.

Ordinarily, the native minerals in which these elements occur constitute a very small fraction of the soil mass and weather so slowly that their absorption by plant life under normal conditions of growth is very low and warrants little attention. Spray residue accumulation in commercial orchard soils, therefore, means the presence of greater than normal amounts of arsenic, lead, and copper in compounds that are more readily soluble in soil solutions than are the native arsenic, lead, and copper-containing minerals.

The authors have thought for many years that there might be possibilities of physiological disturbances in orchard trees almost from the beginning of protective spraying operations. Tree roots, it seemed, would eventually contact abnormal amounts of these chemical elements and suffer from injury

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to their absorbing organs or by internal injury, should there be appreciable absorption. That assumption, it now appears, is not entirely well founded. It prompted, however, the beginning several years ago of a series of chemical investigations on orchard soils and fruits that is aiding in the development of a scientific viewpoint for the consideration of spray residue problems. This paper summarizes certain analytical data on orchard soils and fruits that clarify the nature of soil spray residue problems in commercial orchards.

LITERATURE

Certain contributions to the literature are mentioned because of some influence on the general plan of this investigation or on the interpretation of its analytical results.

Contributions by Headden (13, 14, 15), Morris and Swingle (23), Albert and Paden (1), von Fellenberg (28), Gruner (9), Greaves (8), and Stewart and Smith (27) deal with some phase of arsenic occurrence in the soil and its effect on plant growth. Berry (4) reports on lead nitrate as a source of nitrogen for green plants and notes failure of the metallic element to injure plant root systems. Hammet (10, 11, 12) notes specifically the intake of lead by plant root systems and discusses the manner of its disposal. Skinner (25) deals with the toxic effects on plants of copper accumulations in soils. Mc-Hargue (22), Allison, Bryant and Hunter (3), Lipman (20), Lipman and Mackinney (21), Sommer (26), and Brenchley (5) are concerned with the functioning of copper as an essential element in the growth of higher plants. To these must be added a contribution by Fairhall (6, 7) on lead poison cases occurring in industry and contributions by Kehoe and his associates (16, 17, 18, 19). The last mentioned deal not only with the occurrence of certain non-essential elements in human foodstuffs of plant origin, among which is lead, but with accumulations of those elements in parts of the human body, viz. bones and vital organs, and with their elimination in part via the urine and feces.

It is clear from the literature that the occurrence of these so-called minor chemical elements in soils and the functioning of each in plant growth have been subjects of interest to plant and soil chemists for many years. Realization of the extent to which the elements of inorganic spray mixtures are accumulating in orchard and other soils under long-time protective spraying operations is certain to intensify that interest.

SOIL SPRAY RESIDUE PROBLEMS

This investigation (one of a series) deals with apple and pear orchards only, since apples and pears are given heavier applications of protective sprays than any other orchard fruits. It seeks mainly to determine: the soil zone or layer in commercial orchards in which the arsenic, lead, and copper of spray residues tend to accumulate; some criteria by which to judge normality with respect to the arsenic, lead, and copper content of unsprayed orchard fruits; whether

abnormal soil conditions caused by the accumulations of arsenic, lead, and copper are sharply reflected in the composition of orchard fruits with respect to these chemical elements; wherein lies the greatest threat if any, of inorganic spray residue accumulations in orchard soils to plant and animal welfare.

It is believed that experimental data bearing on these points will aid in clarifying a subject on which commercial orchardists, horticulturists, entomologists, manufacturing chemists, and the consuming public are somewhat confused. Unquestionably, many persons feel that the present-day free use of cumulative inorganic poisons in the growing of foodstuffs is dangerous. Experimentally established facts may allay that suspicion, or they may support it; in the latter case, they may also point the way to the removal of the cause.

<center>EXPERIMENTAL</center>

Selection and preparation of soil and fruit samples

Soil samples for laboratory examination were taken in four of the leading fruit-growing sections of Oregon from areas known never to have been sprayed and from commercial orchard tracts whose spray and cultural history was readily obtainable. It was not always possible to get the two kinds of samples from immediately adjacent areas, and the commercial orchard tracts were sampled more frequently when it became apparent that arsenic, lead, and copper were generally found to be native to all soils, and that the minerals containing them are distributed with fair uniformity throughout the soil mass. The zones sampled have greater significance in the case of the commercial orchard tracts. The second and third zones were arbitrarily chosen, but the top 6–8 inches coincides fairly well with the depth of orchard cultivation and, therefore, with the depth of mechanical mixing of spray residues with soil particles. The distribution of appreciable amounts of spray residues below this layer is the work of some other factor than mechanical mixing. In the orchards, the samples were taken to represent fairly the areas which received the drip or run-off from the foliage and limbs. The sampling tool was a sharp spade. Vertical holes were dug to the desired depth and slices representative of each layer from top to bottom were taken from the sides for the laboratory work.

The soil samples were air-dried, thoroughly mixed, and ground with mortar and pestle until sufficiently fine to pass a 20 or 60 mesh screen. In no case was grinding severe enough to crush small pebbles and fragments of rock. The finer grinding was of samples representing the heavy clay soils. From the sifted soil thus obtained, samples were taken for the quantitative determination of arsenic, lead, and copper.

Fruit samples for laboratory examination were picked from the trees at harvest time. They were taken from trees never sprayed, growing in soils never sprayed, and from trees growing in commercial orchards regularly and

thoroughly sprayed from commencement of bearing. It was impossible to obtain all the samples of unsprayed fruit from areas immediately adjacent to commercial orchard tracts.

The preparation of fruit samples for laboratory work involved careful technic to prevent contamination of the edible portions by skins or any other parts that might carry traces of spray residues. The object was to determine how much arsenic, lead, and copper entering the tree via the rootlets is deposited in the edible portion of the fruit. Samples from the sprayed orchards carried the usual coating of spray mixtures and were more difficult to handle. Preparation involved dipping the samples in petroleum ether, followed by dipping repeatedly in warm, dilute hydrochloric or nitric acid, and rinsing with running water; double paring with an all-steel knife with water rinsing between, or paring from inside out following the breaking open of the fruit by pressure with the fingers; cutting away and discarding of stem ends, calyx ends, cores, and seeds; and drying the finely sliced edible portions in a steam-heated cabinet in large porcelain evaporating dishes. The samples were kept in the dishes awaiting analysis, which followed thorough testing of adapted or devised analytical procedures. Since collection of both fruit and soil samples extended over a period of several years, the three elements were seldom determined on the same sample. Arsenic received earliest attention and copper latest.

Analytical data on soils and fruits

The tabulated data are arranged to show for each element: the soil's native content; in what zone of commercial orchard soils spray residue accumulates; the extent of assimilation by the fruit of apple and pear trees that have only the soil's native mineralized content of that element to draw upon; the extent of assimilation by fruit in commercial orchards.

Arsenic in soils

Analytical data for arsenic in soils were obtained in the early stages of this investigation by the Gutzeit method[3] following wet combustion of the sample with nitric and sulfuric acids. Later, the bromate or distillation method for arsenic,[4] modified by substitution of phenyl hydrazine sulfate for ferrous sulfate, was found to be equally adaptable and more convenient. By this method, distillation of arsenic as $AsCl_3$ followed wet combustion of the sample. The distillate was titrated with $KBrO_3$ solution with methyl orange as indicator. Analytical data by this method only are given in tables 1 and 2.

The native arsenic content of soils representative of extensive orchard tracts before spraying operations is shown in table 1.   Three orchard districts widely separated and different with respect to series and types of soil are represented in the table.   In each district, the series and types chosen are among those predominating.   Arsenic is native in all, with a tendency toward larger amounts in the heavier types.   To the depth sampled, the distribution of native arsenic in any one series is uniform.   This fact makes relatively easy the interpretation of analytical data presented in table 2.

TABLE 1

The native arsenic content of orchard soils

SOIL	ZONE	As$_2$O$_3$ IN DRY SOIL, AVERAGE OF DUPLICATES
	inches	*p.p m.*
1. Gravelly clay loam................	0–15	6.9
	15–30	8.0
2. Clay loam..., ...	0–12	3.9
	12–18	4.8
3. Sandy loam.....	0–12	9.2
	12–16	11.1
4. Clay loam, heavy....	0–12	18.6
	12–16	17.6
5. Adobe, sticky......	0–12	8.1
6. Adobe, sticky..	0– 6	11.9
7. Clay loam, light.	0– 8	17.7
	8–16	14.3
	16–24	11.7
8. Stony clay loam*.	0–20	15.0
	At 20	15.7
	0–20	17.1
9. Sandy loam........	0– 8	6.8
	8–14	4.1
	14–20	5.5
10. Silt loam..........................	0–12	4.8

* Separate samples taken from the same orchard.

Soils numbered 1 to 16, in table 2, represent one large irrigated orchard section in which heavy soils predominate.   Number 17 came from a non-irrigated district in which the rainfall approximates 30 inches annually and where orchard soils range from light clay to fairly heavy clay loams.   Soils 18 to 21 represent a smaller irrigated district in which the soils are of the sandy order.   Number 15 in the first group, although adjacent to irrigated land, has always been "dry farmed."   The letters *A*, *B*, and *C* following the soil numbers are roughly indicative of the maturity of the orchard.   One orchard only could be classed in the *A* or relatively young group.   Those in the *B* group are fully

TABLE 2

Arsenic in the soils of commercial orchards

SOIL		ZONE	As₂O₃ IN DRY SOIL, AVERAGE OF DUPLICATES
		inches	*p.p.m.*
1-*C*	Gravelly clay loam	0– 8	77.8
	Newtown Apples	8–16	8.2
		16–24	6.8
2-*C*	Sandy loam	0– 8	90.0
	Anjou pears	8–16	6.8
		16–24	2.7
3-*B*	Gravelly clay loam	0– 8	87.4
	Bartlett pears	8–16	9.6
4-*C*	Clay loam, heavy	0– 8	61.4
	Comice and Bosc pears	8–16	6.8
5-*C*	Silty clay loam	0– 8	250 0
	Winter Nelis pears	8–16	14.5
		16–24	6.9
6-*B*	Clay adobe	0– 8	82.2
	Bosc pears	8–16	15.0
7-*B*	Clay adobe	0– 8	96.7
	Bosc pears	8–16	26.7
		16–24	14.8
8-*A*	Clay adobe, sticky	0– 8	84.6
	Bartlett pears	8–16	12.3
		16–24	4.1
9-*C*	Clay adobe, sticky	0– 8	580.0
	Anjou pears	8–16	24.6
		16–24	20.5
10-*C*	Gravelly loam	0– 8	23.2
	Winter Nelis pears	8–16	9.6
		16–24	6.8
11-*C*	Clay adobe, black sticky	0– 8	94.3
	Newtown apples	8–16	8.2
12-*C*	Clay adobe, sticky	0– 8	74.1
	Winter Nelis pears	8–16	10.9
		16–24	9.6
13-*C*	Gravelly clay loam	0– 8	120.0
	Newtown apples	8–16	28.7
		16–24	10.9

TABLE 2—*Concluded*

SOIL	ZONE	As₂O₃ IN DRY SOIL, AVERAGE OF DUPLICATES
	inches	*p.p.m.*
14-*C* Clay adobe, black, sticky	0– 8	169.0
Anjou pears	8–16	8.2
15-*C** Sandy loam	0– 8	143.3
Newtown apples	8–16	12.3
16-*B* Clay adobe, Bosc pears	0– 8	92.8
	8–16	2.7
17-*B* Clay loam, light	0– 8	77.0
Wagner apples	8–16	22.5
	16–24	12.3
18-*B* Loam, Jonathan apples	0– 8	57.3
	8–16	6.8
19-*B* Sandy loam	0– 8	57.3
Newtown apples	8–16	12.3
	16–24	13.7
20-*B* Silt loam	0– 8	80 3
Spitzenberg apples	8–16	10.0
	16–24	9.5
21-*B* Sandy loam	0– 8	81.0
Newtown apples	8–16	3.8
	16–24	1.9

* Dry farmed.

matured and producing heavily. The *C* group includes orchards that are among the oldest in the district. All of them have been sprayed for at least 15 years. During this time, the tendency has been toward an increasing number of spray applications yearly and a greater concentration of arsenicals per unit volume of mixture. Although satisfactory duplicate and triplicate determinations of arsenic by the bromate method were made, its reliability for the determination of arsenic in soils was tested also by the recovery of measured amounts of As₂O₃ contained in standard solutions. To a series of four soils ranging from low to high in arsenic content, as determined by distillation and titration, 1 ml. of a standard As₂O₃ solution carrying 0.3241 mgm. of As₂O₃ was added to the usual charge of soil used in the determination. The total arsenic was then determined by distillation. The average recovery for the four determinations was 99.8 per cent. In view of the fact that all errors in manipulation were thrown upon the recovery of the added amounts, this figure is considered highly satisfactory. Therefore, certain conclusions may

be drawn from tables 1 and 2 about the ultimate distribution through the soil profile of added arsenic in the form of spray residue. Whatever may be the variation in total amounts of added arsenic, that element becomes incorporated in orchard soils, irrigated or non-irrigated, only to the depth to which the surface soil is cultivated.

Arsenic assimilation by apples and pears

Destruction of the organic matter in 50-gm. samples of the dried fruit was accomplished by wet combustion with nitric and sulfuric acids. The quantitative determination of the arsenic was made by both the Gutzeit and the

TABLE 3

Arsenic in apples and pears from orchards never sprayed

(Analysis by Gutzeit method)

ORCHARD AND SOIL	FRESH EDIBLE PORTION	
	As$_2$O$_3$, mgm. per kgm. $\times$ 1000	As$_2$O$_3$ grains per lb.
Pear.* Silt loam........................ ...	9.9	.00007
Pear, Pound. Clay loam	28.9	.00020
Pear.* Stony clay loam........................ .	13.1	.00009
Apple.* Clay loam........	2.2	.00002
Apple.* Stony clay loam, old, abandoned orchard.....	3.1	.00003
Apple, Arkansas Black. Stony clay loam..	6.6	.00004
Apple, Gloria Mundy. Clay loam................ ..	10.0	.00007
Apple.* Silty clay loam..................	7.8	.00005
Apple.* Silty clay loam.....................	3.9	.00003
Apple, Newtown. Sandy loam.....................	9.5	.00007
Apple, Leidy. Clay loam	5.2	.00004
Apple.* Silty clay loam...........	9.5	.00007
Apple, seedling. Silt loam..........	23.6	.00017
Average, Pears (3).	17.3	.00012
Apples (10)............................	8.1	.00006
All (13)................................	10.3	.00007

* Variety unknown.

bromate or distillation method, modified as indicated for its use on soils. In tables 3, 4, and 5, the arsenic content, As$_2$O$_3$, is expressed in milligrams per kilogram of the edible portion of fresh fruit $\times$ 1000, and in grains per pound. The latter is included so that comparisons may be made quickly with the legal tolerance for arsenic on the surfaces of fruits entering interstate and foreign trade.

In one sense, the analytical data in table 5 for arsenic by the bromate method are a check on data in table 4. The samples represented are of a later crop and from fewer orchards. All fruits mentioned in table 5, except samples 13, 14, and 15, were taken in 1933 from commercial orchards temporarily aban-

TABLE 4

Arsenic in apples and pears from commercial orchards

(Analysis by Gutzeit method)

ORCHARD AND SOIL	FRESH EDIBLE PORTION	
	As_2O_3, mgm. per kgm. $\times$ 1000	As_2O_3, grains per lb.
Pear, Bosc. Fine sandy loam.........................	53.5	.00038
Pear, Bosc. Silty clay loam	32.3	.00023
Pear, Bosc. Clay adobe............................	20.5	.00014
Pear, Bosc. Clay adobe............................	60.0	.00042
Pear, Bosc. Clay adobe............................	13.4	.00009
Pear, Bosc. Clay adobe........	25.9	.00018
Pear, Bosc. Sandy loam........	9.9	.00007
Pear, Bosc. Silty clay loam.........................	12.6	.00009
Pear, Bosc. Adobe...............	21.7	.00015
Pear, Anjou. Fine sandy loam.....................	29.5	.00020
Pear, Anjou. Silty clay loam...................... .	16.6	.00012
Pear, Anjou. Clay loam..........	71.0	.00050
Pear, Anjou. Clay adobe...........................	47.0	.00033
Pear, Anjou. Clay loam...........................	20.4	.00014
Pear, Anjou. Fine sandy loam........	16.9	.00012
Pear, Anjou. Clay adobe	11.7	.00008
Pear, Anjou. Silty clay..	7.2	.00005
Pear, Anjou. Clay adobe..........................	19.1	.00013
Pear, Anjou. Loam........	3.7	.00003
Apple, Spitzenberg. Sandy loam	47.0	.00033
Apple, Yellow Newtown. Sandy loam..	53.3	.00037
Apple, Yellow Newtown. Silt loam..	63.0	.00044
Apple, Yellow Newtown. Loam.......	11.1	.00008
Apple, Ortley. Loam.......	10.6	.00007
Apple, Yellow Newtown. Silt loam.................	9.9	.00007
Apple, Spitzenberg. Silt loam	17.3	.00012
Apple, Yellow Newtown. Sandy loam..	35.0	.00024
Apple, Delicious. Sandy loam.......................	39.0	.00027
Apple Spitzenberg. Sandy loam.....................	23.0	.00016
Apple, Arkansas Black. Sandy loam....	25.0	.00018
Apple, Spitzenberg. Sandy loam.....	65.0	.00045
Apple, Jonathan. Sandy loam.....................	103.0	.00072
Gutzeit method:		
Averages, Pears (19)...................	26.0	.00018
Apples (13)................	38.6	.00027
All (32).................................	31.1	.00022
Bromate method (table 5):		
Averages, Pears (15)...........................	41.5	.00029
Apples (5)............................	37.8	.00026
All (20)............................	40.6	.00026

doned. Since they received no spray covering whatever during growth and are among the higher ones in arsenic content, the analyses show that spray residues do not appreciably penetrate the skins of fruit. The significance of these data will be discussed later in connection with similar data for lead and copper.

TABLE 5

Arsenic in apples and pears from commercial orchards temporarily abandoned

(Analysis by bromate method)

ORCHARD AND SOIL	FRESH EDIBLE PORTION	
	As₂O₃, mgm. per kgm. × 1000	As₂O₃, grains per per lb.
1. Pear, Bartlett. Fine sandy loam.....	68 0	.00048
2. Pear, Bosc. Fine sandy loam....	18.7	.00013
3. Pear, Bosc. Fine sandy loam.	39.3	.00028
4. Pear, Bosc. Fine sandy loam.	31.7	.00022
5. Pear, Anjou. Fine sandy loam..	39.2	.00027
6. Pear, Anjou. Fine sandy loam......	90.2	.00063
7. Pear, Winter Nelis. Fine sandy loam	59 4	.00042
8. Pear, Anjou. Fine sandy loam	113.5	.00079
9. Pear, Bartlett. Clay adobe.	38.9	.00027
10. Pear, Bosc. Clay adobe.	5.3	.00004
11. Pear, Bosc. Clay adobe	29.6	.00021
12. Pear, Comice. Clay adobe.......	47.5	.00033
13. Pear, Bartlett. Packing house assortment......	23 1	.00016
14. Pear, Bosc. Packing house assortment.	7 7	.00005
15. Pear, Anjou. Packing house assortment...... ..	11 6	.00008
16. Apple, Newtown. Clay adobe..........	4.3	.00003
17. Apple, Newtown. Clay adobe	12.9	.00009
18. Apple, Newtown. Gravelly clay loam	110.0	.00077
19. Apple, Tompkins King. Clay loam.	39.5	.00024
20. Apple, Yellow Newtown. Clay loam...	27.3	.00019
Bromate method:		
Averages, Pears (15)	41.5	.00029
Apples (5)...............	37 8	.00026
All (20)...	40.6	.00028
Gutzeit method (table 4):		
Averages, Pears (19)...	26.0	.00018
Apples (13).................... .	38.6	.00027
All (32)........................	31.1	.00022

Lead in soils

The literature shows that not until recently has lead been accepted as a normal component of arable soil. In this work, lead was found in all soils examined specifically for it.

For the lead determination in soils, it was necessary to devise an analytical procedure that would prove dependable for both the minute amounts found

in soils never sprayed and for the much larger amounts in the soils of commercial orchards. The analytical procedure outlined here was finally employed.

(*a*) Destruction of organic matter: 2½ gm. of soil in a silica dish are heated for 2 hours in a muffle furnace maintained very close to 500°C.

(*b*) Extraction of ignited residues: Three solvents are used in the order named:—1 + 1 hydrochloric acid, a mixture of 1 + 4 hydrochloric acid and 20 per cent citric acid, and 40 per cent ammonium acetate solution.

(*c*) Separation of dissolved lead from other elements· The mixture of extracts obtained under (*b*), with citric acid added, is made alkaline with ammonium hydroxide and, after addition of potassium cyanide solution, is repeatedly shaken to obtain the complete extraction of the lead with a chloroform solution of dithizone (diphenyl-thiocarbazone, Ph N:NCS· NHNH Ph).

(*d*) Determination of lead: The chloroform-dithizone solution of lead is first evaporated to dryness on the steam bath, and the dithizone destroyed with nitric acid. The lead in 2 per cent nitric acid solution is then electrolytically deposited as PbO_2 on a rotating platinum gauze anode from which it is dissolved by a mixture of sodium acetate and acetic acid. The

TABLE 6

The native lead content of orchard soils

SOIL	ZONE	Pb IN DRY SOIL, AVERAGE OF DUPLICATES
	inches	*p.p.m.*
Sandy loam....	0– 8	9.3
Sandy loam...	8–16	8 2
Clay loam..	0–20	16.8
Fine sandy loam......	0–14	7.6
Clay, light.............	0–16	15.7

addition of potassium iodide solution and the titration of the iodine set free with 0.0025 *N* sodium thiosulfate delivered from a microburette calibrated in 0.01 ml. gives the basis for the calculation of the weight of lead carried by the soils of the orchards sampled.

The samples were tested in duplicate with closely agreeing results but, to test further the reliability of this application to soils of the dithizone extraction method for lead, a series of recovery determinations was made. From 0.3 to 0.6 mgm. of lead in standard lead nitrate solution was added from a microburette to the weighed portions of soil just before placing in the furnace. After this, all operations were identical with those followed on the samples before the addition of lead nitrate. In this series of 10 soils, ranging in texture from loam through sandy loam to heavy clay, the average recovery of added lead was 95.1 per cent. The tendency is plainly toward under, rather than over, determination of lead by this method. The native lead content of typical orchard soils is shown in table 6. The lead content of commercial orchard soils following periods of protective spraying operations ranging in duration from 15 to 25 years is shown in table 7. The surface localization of the lead added is apparent.

TABLE 7

Lead in the soils of commercial orchards

SOIL AND ORCHARD		ZONE	Pb IN DRY SOIL, AVERAGE OF DUPLICATES
		inches	*p.p.m.*
1-*C*	Sandy loam	0– 8	215.6
	Anjou pears	8–16	16.3
		16–24	10.3
2-*C*	Gravelly clay loam	0– 8	233.2
	Newtown apples	8–16	16.3
		16–24	14.1
3-*B*	Gravelly clay loam	0– 8	184.1
	Bartlett pears	8–16	28.2
4-*C*	Clay adobe	0– 8	96.5
	Comice pears	8–16	15.2
		16–24	15.2
5-*B*	Clay adobe	0– 8	266.0
	Bosc pears	8–14	45.5
6-*A*	Clay adobe, heavy	0– 8	313.0
	Bartlett pears	8–16	28.2
		16–24	10.8
7-*C*	Clay adobe, heavy	0– 8	1,383.0
	Anjou pears	8–16	35.8
		16–24	17.3
8-*C*	Clay adobe, heavy	0– 8	216.6
	Newtown apples	8–16	19.5
		16–24	11.9
9-*B*	Gravelly clay loam	0– 8	66.1
	Winter Nelis pears	8–16	15.7
		16–24	16.3
10-*C*	Clay adobe, heavy	0– 8	279.4
	Bosc pears	8–16	29.2
		16–24	24.9
11-*C*	Clay adobe, heavy	0– 8	209.0
	Winter Nelis pears	8–16	19.5
		16–24	22.8
12-*C*	Clay adobe	0– 8	309.0
	Newtown apples	8–16	88.9
		16–24	21.7

TABLE 7—*Concluded*

SOIL AND ORCHARD	ZONE	Pb IN DRY SOIL, AVERAGE OF DUPLICATES
	inches	*p.p.m.*
13-C Clay adobe, heavy	0– 8	348.0 •
Anjou pears	8–16	21.7
	16–24	17.3
14-C* Sandy loam	0– 8	347.0
Newtown apples	8–16	16.3
	16–24	13.0
15-B** Clay, light	0– 8	150.0
Wagner apples	8–16	14.1
	16–24	14.1
16-C Sandy loam	0– 8	121.3
Spitzenberg apples	8–16	15.2
	16–24	10.9
17-C Sandy loam	0– 8	30.3
Jonathan apples	8–16	8.7
	16–24	6.5
18-C Sandy loam	0– 8	127.8
Jonathan apples	8–16	7.6
	16–24	7.6

* Dry farmed.
** Corresponds to 17, table 2.

The letters A, B, and C following the soil numbers in table 7 are, as in table 2, roughly indicative of orchard maturity. In most of the orchards, the surface soils were fairly dry at the time of sampling. For that reason, in spite of all precaution to prevent co-mingling of soil from different zones in sampling, it is probable that contamination was not prevented in all cases. This partially explains what may seem unreasonably high results for lead in the second layer of four of the orchard soils, viz. $5\,B$, $6\,A$, $7\,C$, and $12\,C$. Table 7 indicates that lead from spray residues, like arsenic, is distributed in the profiles of these orchard soils only to the depth it is carried mechanically by the tools of orchard cultivation: the first 6–8 inches.

Lead assimilation by apples and pears

The presence of lead in soils indicates the probability of its movement into the trees and its deposition, to some extent at least, in the tissues of the fruit. By development of a special technic based on principles of recommended microchemical procedure for the detection of minute amounts of lead (24), the black cubical crystals of triple nitrite of potassium, copper, and lead,

$2KNO_2 \cdot Cu(NO_2)_2 \cdot Pb(NO_2)_2$, have been produced repeatedly from the ash of apple and pear twigs and from the ash of their fruits. Typical crystals are shown in plate 1. Careful control of the conditions of ashing and negative blank tests on reagents leave no doubt regarding the origin of the lead. It came from the soil via the root systems.[5]

In principle, the quantitative procedure perfected for the determination of lead in soils was applicable to the determination of lead in fruit tissues. Fifty grams of dried fruit flesh, corresponding roughly to three-quarters of a pound of fresh flesh, were ashed in platinum dishes by very slow combustion in a muffle furnace maintained at 400°C. The lead was leached from the ash

TABLE 8

Lead in apples and pears from orchards never sprayed

ORCHARD AND SOIL	FRESH EDIBLE PORTION	
	Pb, mgm per kgm. × 1000	Pb, grains per lb.
Pear, Pound. Stony clay.........................	46	.00032
Pear, Bartlett. Fine gravelly loam	86	.00060*
Pear, Pound. Stony clay.........................	48	.00034
Pear, variety unknown. Clay loam................	56	.00039*
Pear, Winter Nelis. Stony clay....	172	.00121
Apple, Tolman Sweet. Stony clay.................	139	.00098
Apple, variety unknown. Clay loam..............	121	.00084*
Apple, Bellflower. Stony clay.....	130	.00091
Apple, Newtown. Clay loam...	270	.00189*
Apple, Newtown. Stony clay.....	73	.00051
Apple, Bellflower. Fine gravelly loam....	95	.00067*
Apple, Bellflower. Clay loam....	216	.00151*
Apple, Newtown. Silt loam...........	34	.00024*
Averages, Pears (5).....................	82	.00057
Apples (8).............................	135	.00094
All (13)..	114	.00080

* Average of two or more determinations.

by the same series of solvents, and from there on the analytical procedure was the same as for lead in soils, i.e., extraction by dithizone reagent, electrolytic deposition, and titration. The analytical data obtained by this method are recorded in tables 8 and 9.

The analytical procedure followed was relied upon to measure accurately

[5] Equally conclusive proof of the presence of lead in edible portions of apples and pears came from qualitative spectrographic analysis of four lots of ash from apple and pear flesh. These analyses indicated, too, the presence of certain other non-essential elements, viz. aluminum, barium, bismuth, nickel, rubidium, silver, strontium, titanium, and vanadium. The analyses were obtained through the courtesy of the Bausch and Lomb Optical Company of Rochester, New York.

TABLE 9

Lead in apples and pears from commercial orchards

ORCHARD AND SOIL	FRESH EDIBLE PORTION	
	Pb, mgm. per kgm. × 1000	Pb, grains per lb.
Pear, Bosc. Sandy loam...........................	89	.00062
Pear, Bosc. Sandy loam...........................	40	.00028
Pear, Bartlett. Gravelly loam....................	284	.00199*
Pear, Anjou. Sandy loam.........................	80	.00056
Pear, Anjou. Sandy loam.........................	68	.00048
Pear, Bosc. Gravelly loam.......................	205	.00143*
Pear, Winter Nelis. Sandy loam..................	107	.00074
Pear, Anjou. Sandy loam	105	.00073
Pear, Anjou. Fine sandy loam	211	.00148*
Pear, Bartlett. Clay adobe..........	169	.00119
Pear, Bartlett, packing house cross section	241	.00168
Pear, Bosc, packing house cross section..............	113	.00070
Pear, Anjou, packing house cross section..............	148	.00103
Pear, Winter Nelis. Fine sandy loam..............	78	.00054*
Pear, Bosc. Clay adobe..	76	.00053
Pear, Winter Nelis. Clay adobe.	95	.00066
Pear, Bosc. Gravelly loam.....	123	.00086
Pear, Bosc. Clay loam...........................	131	.00092
Pear, Anjou. Clay adobe.............	90	.00063
Pear, Anjou. Fine sandy loam	138	.00097*
Pear, Comice. Clay adobe	89	.00062
Pear, Comice. Clay adobe......................	57	.00040
Pear, Bosc. Silt loam........................ ...	185	.00130*
Pear, Anjou. Clay adobe..	44	.00031
Pear, Anjou. Sandy loam.................	48	.00034
Pear, Anjou. Silt loam.................	263	.00185*
Pear, Bosc. Clay adobe.........	82	.00057
Apple, Newtown. Clay adobe	69	.00048
Apple, Jonathan. Silt loam.	225	.00159
Apple, Newtown. Gravelly clay loam.	115	.00080
Apple, Winesap. Gravelly silt loam..................	63	.00044*
Apple, Tompkins King. Light clay.................	49	.00034
Apple, Newtown. Silt loam....................	100	.00070*
Apple, Northern Spy. Light clay..............	79	.00056
Apple, Spitzenberg. Silt loam..	97	.00068*
Apple, Grimes Golden. Light clay.................	173	.00120
Apple, Spitzenberg. Silt loam..................... ..	63	.00045*
Apple, Newtown. Sandy loam......................	69	.00048
Apple, Newtown. Silt loam........................	39	.00028*
Apple, Newtown. Sandy loam......................	64	.00045
Apple, Rome Beauty. Gravelly silt loam.............	94	.00066*
Apple, Ortley. Sandy loam..........................	29	.00021
Apple, Winesap. Gravelly silt loam.................	128	.00090*
Apple, Spitzenberg. Sandy loam....................	17	.00012

* Average of two or more determinations.

TABLE 9—*Concluded*

ORCHARD AND SOIL	FRESH EDIBLE PORTION	
	Pb, mgm. per kgm. × 1000	Pb, grains per lb.
Apple, Winesap. Gravelly silt loam..................	185	.00130*
Apple, Seedling. Sandy loam.......................	48	.00034*
Apple, Newtown. Clay adobe......................	41	.00029
Averages, Pears (27)............................	124	.00087
Apples (20)............................	87	.00061
All (47)................................	109	.00076

in the 50 gm. of material used amounts of lead ranging between 0.02 and 0.10 mgm.   As a test of reliability, recovery data were obtained as the work progressed.   Additions were made from a micro-burette of 0.2 to 0.3 mgm. of lead in standard lead nitrate solution to 50-gm. portions of the dried fruits. These portions were then treated in all ways, as were the samples to which no lead had been added.   For a total of 23 such determinations, the average recovery of added lead was 96.7 per cent.

Copper in soils

A study of the literature indicates that copper is now generally considered a normal component of arable soils.   In this work, copper was found in unsprayed soils in amounts ranging from 1 to 34 p.p.m.   Copper compounds are less commonly used in orchard spraying operations than are those of arsenic.

After somewhat unsatisfactory experience with recommended analytical procedures for small amounts of copper, a method was developed that proved satisfactory for the determination of copper in both soils and fruit.   The several steps are:

(*a*) Oxidation of organic matter: 5 gm. portions of soil in 250 ml. beakers are digested at 200°C. with sulfuric and perchloric acids, 1 and 2 by volume, to disappearance of fumes.

(*b*) Solution and first precipitation: Following digestion of the oxidized portion with boiling concentrated hydrochloric acid, the insoluble material is filtered onto a thin layer of asbestos in a Gooch crucible and washed with water.   Filtrate and washings are next concentrated to a volume of 10 ml. and addition made of 15 ml. of 20 per cent tartaric acid solution.   Neutralization is then effected with concentrated ammonium hydroxide and re-acidification with 1 ml. of concentrated sulfuric acid.   Following the addition of 10 ml. of 20 per cent sodium sulfite solution, the copper is precipitated at boiling temperature by the addition of 1 ml. of 20 per cent potassium thiocyanate solution.

(*c*) Electrolytic deposition: The precipitate of cuprous thiocyanate, after standing from 8 to 10 hours, is filtered onto sintered glass and washed with potassium thiocyanate and tartaric acid solution, 0.1 per cent each in water, and finally with a volume of water not exceeding 10 ml.   Solution is then made of the cuprous thiocyanate in 5 ml. of hot concentrated nitric acid.   Following the addition of 1 ml. of concentrated sulfuric acid, dilution to 200 ml., short-time boiling, and addition of 0.5 gm. of urea, the copper is deposited on a rotating platinum gauze cathode by approximately 20 minutes of electrolysis at 70° C.   The current used is 300 milliamperes at 3 volts.

TABLE 10
The native copper content of orchard soils

SOIL	ZONE	Cu IN DRY SOIL, AVERAGE OF DUPLICATES
	inches	*p.p.m.*
1. Silt loam...............................	0–15	16.0
2. Silt loam...............................	0–15	21.2
3. Silt loam...............................	0–15	20.6
4. Silt loam...............................	0–15	10.6
5. Silt loam...............................	0–15	1.2
6. Clay loam...............................	0– 8	33.9
	8–16	34.2
	16–24	33.6
7. Stony clay loam.........................	0–20	4.5
8. Gravelly loam...........................	0– 8	18.1
	8–16	17 5
	16–24	15.0
9. Fine gravelly loam......................	0– 8	8.9
	8–16	11.6
	16–24	8.1
10. Clay loam..............................	0–12	11.9

TABLE 11
Copper in the soils of commercial orchards

SOIL AND ORCHARD (IRRIGATED)	ZONE	Cu IN DRY SOIL, AVERAGE OF DUPLICATES
	inches	*p.p.m.*
1. Silt loam. Bosc pears..................	0– 8	24.1
	8–16	13.0
	16–24	9 1
2. Silt loam. Bartlett pears..............	0– 8	55 6
	8–16	13.7
	16–24	15.3
3. Silt loam. Newtown apples.............	0– 8	52.5
	8–16	23.8
	16–24	18.8
4. Silt loam. Newtown apples.............	0– 8	68.5
	8–16	33.1
	16–24	24.1
5. Silt loam. Newtown apples.............	0– 8	143.8
	8–16	41.2
	16–24	28.8
6. Silt loam. Spitzenberg apples..........	0– 8	83.7
	8–16	5.3
	16–24	3.2
7. Clay loam. Wagner apples..............	0– 8	80.0
	8–16	27.6
	16–24	29.4

(d) Final solution and titration. Solution of the electrolytically deposited copper is effected with hot, dilute, nitric acid, which then is concentrated in a porcelain casserole to a volume of 1 ml., neutralized with ammonium hydroxide, and reacidified with acetic acid. Addition of 0.25 gm. of potassium iodide, dilution to 30 ml., and titration of the liberated iodine with 0.005 N sodium thiosulfate, completes the analytical work.

By this procedure, the analytical data for copper in tables 10, 11, 12, and 13 were obtained.

In tables 10 and 11 are given the analytical data for copper in soils never sprayed or dusted with copper compounds, and for the copper in commercial orchard soils sprayed-over with bordeaux or other copper-containing mixture. In these tables, three orchard sections only are represented, two of them irrigated. From the analytical data in table 11, it is apparent that there is the same tendency for spray residue copper to remain localized in the surface layer of soil that has been noted for both arsenic and lead. In testing the reliability of the procedure developed here for the determination of small amounts of copper in soils, recovery data were obtained for 1 mgm. of copper in standard copper sulfate solution added to 5-gm. portions of soil previous to wet combustion. The average recovery on 12 samples was 97.8 per cent.

Copper assimilation by apples and pears

Abundant qualitative proof of the presence of copper in these fruits developed in the course of the qualitative tests for lead. It was seldom necessary to *add* a solution of copper salt in those tests to produce the crystals of triple nitrite of potassium, copper, and lead.

The quantitative analytical procedure for copper in fruits was varied in two particulars only from that devised for copper in soils. The dried flesh, 25 gm., was dry-ashed in silica dishes in a muffle furnace in two stages. The first was over-night combustion at 400°C. The incompletely burned residue was leached once with 5 ml. of 1 + 1 nitric acid. The leached residue was then reheated, 2–3 hours, at 400°C. to complete disappearance of carbon. The leachings from this ash with 1 + 1 nitric acid were added to those from the first and, with the addition of 1 ml. of concentrated sulfuric acid, were diluted to 200 ml., from which copper was deposited electrolytically without the intervening precipitation of cuprous thiocyanate.

In table 12 are given the analytical data for copper found in apples and pears picked from trees whose sole source of copper was the native mineralized form of that element. In table 13 are shown corresponding data for fruits picked in commercial orchards whose spray programs include the distribution of some form of copper, usually bordeaux mixture. The average recovery of 0.2 mgm. to 0.5 mgm. of copper, added to eleven 25-gm. portions of fruit in practically the same manner as it had been to the soil samples as a test of reliability of analytical procedure, was 95.7 per cent. It will be noted that the copper content of apples and pears is substantially higher than is that of either arsenic or lead.

TABLE 12

Copper in apples and pears from orchards never sprayed

ORCHARD AND SOIL	FRESH EDIBLE PORTION	
	Cu, mgm per kgm. × 1000	Cu, grains per lb.
Apple, Newtown. Clay loam	708	.00496
Apple, Newtown. Clay loam	644	.00451
Apple, Newtown. Clay loam	217	.00152
Apple, Newtown. Silt loam	275	.00194
Apple, Bellflower. Stony clay loam	458	.00321
Apple, Bellflower. Fine gravelly loam	682	.00477
Apple, Winesap. Gravelly loam	410	00287
Apple, Winesap. Gravelly loam	631	.00442
Apple, Winesap. Gravelly loam	363	.00254
Pear, Bartlett. Fine gravelly loam.	901	.00631
Pear, Bartlett. Fine gravelly loam	797	.00558
Pear, Anjou. Silt loam	1033	.00724
Pear, Anjou. Silt loam	649	.00454
Pear, Bosc. Silt loam	960	.00672
Pear, Bosc. Gravelly loam	929	.00650
Pear, Pound. Stony clay loam.	573	.00401
Pear, Pound. Stony clay loam	545	.00382
Averages, Apples (9)	487	.00342
Pears (8)	798	.00559
All (7)	634	.00444

TABLE 13

Copper in apples and pears from commercial orchards

ORCHARD AND SOIL	FRESH EDIBLE PORTION	
	Cu, mgm. per kgm × 1000	Cu, grains per lb.
Apple, Newtown. Silt loam.	669	.00468
Apple, Newtown. Silt loam	267	.00187
Apple, Newtown. Sandy clay loam	365	.00256
Apple, Newtown. Clay adobe.	590	.00413
Apple, Spitzenberg. Silt loam	544	.00381
Apple, Spitzenberg. Silt loam	369	.00258
Apple, Spitzenberg. Sandy clay loam	589	.00412
Apple, Spitzenberg. Sandy loam	561	.00392
Apple, Jonathan. Silt loam	540	.00378
Pear, Anjou. Silt loam	928	.00650
Pear, Anjou. Silt loam	1682	.01179
Pear, Anjou. Sandy loam	1064	.00745
Pear, Anjou. Clay adobe	1450	.01014
Pear, Bartlett. Silt loam.	1006	.00704
Pear, Bosc. Silt loam	1184	.00830
Pear, Bosc. Gravelly loam	1443	.01011
Pear, Bosc. Sandy loam	1520	.01063
Pear, Winter Nelis. Silt loam	1541	.01080
Averages, Apples (9)	499	.00349
Pears (9)	1313	.00920
All (18)	906	.00635

Although there is wider significance in the foregoing analytical data, only that which is immediately and generally applicable to conditions prevailing in commercial orchards will be discussed here.

Arsenic, lead, and copper are normal components of orchard soils and fruits

Examination of the analytical data in tables 1, 6, and 10 confirms the general understanding that arsenic, lead, and copper may be accepted as normal components of arable soil and hence are to be found in fairly even distribution in mineralized forms in the soils of commercial orchards. Therefore, spray residue accumulations from the use of lead arsenate and the copper compounds merely increase the soils' native content of those elements; they do not introduce something new. This is true also if other arsenicals have been substituted for lead arsenate in the spray programs of commercial orchards, since calcium, iron, magnesium, manganese, and possibly zinc are likewise native in mineralized form in all soils. It is probable, therefore, in the light of an earlier statement, that orchard trees will absorb through their root systems some of this native arsenic, lead, and copper and will build some of each element into the tissues of their various parts, including fruits. It is apparent that tree root systems must contact these mineralized elements, and it is probable that every soil mineral is soluble to some degree in soil solutions. The analytical data in tables 3, 8, and 12 are proof that some breakdown of arsenic, lead, and copper-containing minerals is normal in all soils thus far studied in this work. Apple and pear trees growing in soils never sprayed have absorbed through their root systems at least appreciable amounts of arsenic, lead, and copper, as one may note from amounts found by analysis in their fruits. In the edible portion of fresh fruits, arsenic as trioxide ranges between 2 and 29 parts per billion, lead between 34 and 270 parts per billion, and copper between 217 and 1,033 parts per billion. These data indicate that arsenic, lead, and copper are also normal components of apples and pears. The amounts found, however, are only approximate for the fruits of trees grown in soils never sprayed. It was always necessary in this work, in getting fruits from those trees, to make use of abandoned orchards. The growth of trees and fruits in them was seldom normal because of neglect of cultivation and irrigation. Since both operations are conducive to mineral weathering and hence to the availability of all inorganic elements to root absorption, there is reason to believe that if unsprayed trees were in all respects—pruning, cultivation, irrigation, and cover cropping—treated as are commercial orchards of apples and pears, their fruits would run somewhat higher in arsenic, lead, and copper than did the samples whose analyses are reported in tables 3, 8, and 12.

Localization of spray residue accumulation in orchard soils

The toxicity to plant and animal life of the principal elements used in protective sprays and dusts is generally known for the somewhat artificial conditions under which it has been studied. As a result, some orchardists probably suspect that they may be creating dangerous conditions in their own orchards by the seasonal spreading of large amounts of those same compounds. That commercial orchard trees with 30 to 40 years of intensive spray history have not yet shown arsenical, lead, or copper injury is especially noteworthy. It is explainable now on the basis of the analytical data reported in tables 2, 7, and 11.

It is apparent from these data that there is surface localization of spray residue compounds. They cannot penetrate the soil mass unaided. Each remains permanently in the 6 to 8 inches of soil that is cultivated. The surface 8 inches of a commercial orchard soil receives with each year of spraying approximately $1\frac{1}{2}$ times its original content of both arsenic and lead (copper in a much lower ratio if bordeaux or other copper-containing fungicide is used) in forms that are more readily available for plant root absorption than are the native mineralized forms of those elements. Notwithstanding many years of effective spraying operations, those horizons of commercial orchard soils below the maximum depth cultivated are practically unchanged from their native condition in their content of arsenic, lead, and copper. Even in the oldest of commercial orchards there is a wide margin of safety to the trees in this situation. Unfortunately, however, it forbodes danger in another direction.

Arsenic, lead, and copper in fruit of commercial orchards

Tables 4, 5, 9, and 13 show that apple and pear trees in commercial orchards deposited in their fruits during the season in which they were sampled between 4 and 113 parts per billion of arsenic trioxide, between 17 and 284 parts per billion of lead, and between 267 and 1,682 parts per billion of copper. When these data are compared with like data in tables 3, 8, and 12, the conclusion is that there is small difference between absorption and deposition in their fruits of arsenic, lead, and copper by trees in commercial orchards and trees growing in soils never sprayed. It had been tacitly assumed that fruit trees in commercial orchards would respond promptly to increases in soil accumulation of spray residue compounds by increased intake and deposition in their tissues of these and any other chemical elements involved in spray residues. An explanation of the disagreement between our findings and this assumption has probably been given by Aldrich, Work, and Lewis (2) in their researches in pear tree root distribution. They found that although 35 per cent of the total root absorbing system is concentrated in the top foot of soil, a much smaller portion, approximately 7 per cent, is to be found above the 6-inch depth. Absorption by the root systems of commercial orchard trees and deposition in

their fruits of arsenic, lead, and copper are in large measure, then, limited to the same source of those elements as are available to trees in soils never sprayed; i.e., to the native soil mineralized forms.

This is not entirely true, however, because there is at times deep cracking of soil structure, particularly in soils of close-textured types. Into the cracks, to depths generally much greater than 8 inches, some surface soil and its accumulation of spray residues is carried mechanically. Also, however "insoluble" or firmly "fixed" spray residue compounds may become in the soil complex of the surface horizon, they are not entirely unaffected by the solvent effect of rain and irrigation water. The presence of arsenic, lead, and copper in drainage water from commercial orchard soils is readily demonstrable. These facts may be taken as proof that tree roots in commercial orchard soils must have somewhat greater contact than those in unsprayed orchards with readily available sources of arsenic, lead, and copper. But, regardless of the cause of the sub-surface presence of spray residue elements in commercial orchard soils, it is evident from tables 2, 7, and 11 that such amounts are low in comparison with those in the surface layer of 8 inches. From analytical data in tables 4, 5, 9, and 13, it is evident, too, that the deposition in the fruits of arsenic, lead, and copper is not greatly increased through root absorption.

At present, it is not definitely known what response orchard trees might make in root absorption and deposition in their fruits of arsenic, lead, and copper to an even distribution of accumulated spray residues to the depth penetrated by their root systems.

Dangers inherent in spray residue accumulations

However important and reassuring the facts just stated may be, it does not follow that the conditions gradually created in commercial orchard soils through spraying operations carry no threat of danger to orchard or human welfare. Few owners would take that position. On the contrary, close observers will agree that, simultaneously with the development of proof of the surface localization of accumulated spray residues, increasingly frequent instances of complete failure in growth on the part of cover crops or of manifestations of acute disturbance at some stage of growth have been reported. Since cover crops usually are surface-feeding crops, it may be that the elements of spray residue accumulations are partly the cause. Cover crops are necessary for the maintenance of soil tilth. A difficult situation has been developed if arsenic, lead, and copper are directly or indirectly the cause of cover crop failure. How then to rid the surface soil of those elements becomes the problem.

The certainty that commercial orchards must give way in rotation to other food and forage crops causes other problems to arise. In the main, these crops are surface feeders. A much larger part of their root systems than is the case with orchard trees must contact during the entire period of growth the portion of the soil mass in which the bulk of the soil accumulation of in-

organic spray residues is concentrated. Even if such accumulations will not be so great as to prevent the growth of surface-feeding crops, prevailing conditions during growth will result in absorption and deposition in edible parts of the maximum assimilable amounts of a number of toxic inorganic chemical elements. What is, or will be, the actual response of those crops to these abnormal soil conditions in their intake especially of arsenic, lead, and copper? The basis for a definite answer is still to be established. Experimental evidence thus far obtained, however, warrants the statement that the tendency is toward a general and substantial increase in root absorption of these elements, and that assimilation of each far in excess of the normal by aerial and edible parts may reasonably be expected. And then, what will be the reaction of the animal body when it, too, is limited by force of circumstances to rations that are substantially richer in arsenic, lead, and copper than were any on which it had previously fed? Again, a definite answer, based on experimental evidence, cannot yet be given. The literature, however, indicates the necessity of thorough experimental consideration in the interest of both human and livestock welfare.

SUMMARY

This investigation was directed toward a better understanding of the significance of spray residue accumulations in commercial orchard soils than has prevailed generally. Such accumulations are inevitable if orchard trees and fruits are protected adequately against various insect pests and fungous diseases, for waste of spray materials in spraying operations is large and unavoidable.

Analytical data on commercial orchard soils and fruits indicate plainly that orchard welfare is not directly affected by soil spray residue accumulations, however long the period of accumulation. Explanation of these facts lies in the proof of surface-soil localization of the compounds of spray residue accumulation. In the main, those compounds penetrate the soil mass only to such depth as they are carried mechanically by orchard tools of cultivation: approximately 6–8 inches. Since tree root systems in the main distribute themselves in lower horizons of the soil profile, the major part of their absorbing portions do not contact the portion of the soil mass in which the increase of arsenic, lead, and copper-containing minerals occurs. Therefore, tree roots escape injury and their absorbing organs have little chance to send more than normal amounts of those elements to the fruits and other aerial parts for assimilation.

Here and there in orchard districts there is increasing difficulty in the growing of cover crops that are essential in maintaining soil tilth. Such crops either fail in growth altogether or make only scanty growth. It has been suggested that soil accumulation of spray residues have sometimes caused these failures. Since cover crops in general are surface-feeding crops, and the surface localization in orchard soils of spray residue accumulation has been shown,

this suspicion may be well founded, even though the suspected relationship of cause and effect proves eventually to be indirect only. Further investigation is necessary to clear up this point.

Also, in time commercial orchards will give way in rotation to the annual surface-feeding food and forage crops. The root systems of these crops must contact during their entire growing period that part of the soil mass in which lies many years' accumulations of inorganic spray residues. If the situation is not so bad as to prevent growth altogether, it will cause root absorption and assimilation in edible parts by these plants of maximum amounts of chemical elements toxic in the animal body. It is not yet definitely known how surface-feeding plants will respond under these conditions in the matter of intake and assimilation. The limited amount of evidence, however, points to both increased root absorption of arsenic, lead, and copper and increased assimilation of each by aerial and edible parts. In food and feedstuffs thus enriched in chemical elements that are definitely harmful to the animal organism, there probably lies whatever threat there is to human and livestock welfare in the accumulation of inorganic spray residue compounds in orchard soils.

REFERENCES

(1) ALBERT, W. B., AND PADEN, W. R. 1931 Calcium arsenate and unproductiveness in certain soils. *Science* 73: 622.

(2) ALDRICH, W. W., WORK, R. A., AND LEWIS, M. R. 1935 Pear root concentration in relation to soil-moisture extraction in heavy clay soil. *Jour. Agr. Res.* 50: 975–988.

(3) ALLISON, R. V., BRYANT, O. C., AND HUNTER, J. H. 1927 The stimulation of plant response on the raw peat soils of the Florida Everglades through the use of copper sulfate and other chemicals. *Florida Agr. Exp. Sta. Bul.* 190: 33–80.

(4) BERRY, R. A. 1924 The manurial properties of lead nitrate. *Jour. Agr. Sci.* 14: 58–65.

(5) BRENCHLEY, WINIFRED E. 1914 Inorganic Plant Poisons and Stimulants. Cambridge University Press, Cambridge.

(6) FAIRHALL, L. T. 1922 Lead studies. Estimation of minute amounts of lead in biological material. *Jour. Indus. Hyg.* 4: 9.

(7) FAIRHALL, L. T. 1926 Chromate method for minimal amounts of lead in fecal specimens. *Indus. and Engin. Chem.* 18: 431–432.

(8) GREAVES, J. E. 1934 The arsenic content of soils. *Soil Sci.* 38: 355–361.

(9) GRUNER, H. 1911 Arsenic content of soils of Reichenstein. *Landw. Jahrb.* 40: 517–557.

(10) HAMMET, F. A. 1928 Studies in the biology of metals. *Protoplasma* 4: 183–191.

(11) HAMMET, F. A. 1928 Studies in the biology of metals. *Protoplasma* 5: 134–141.

(12) HAMMET, F. A. 1928 Studies in the biology of metals. *Protoplasma* 5: 535–562.

(13) HEADDEN, WM. P. 1908 Arsenical poisoning of fruit trees. Colo. Exp. Sta. Bul. 131.

(14) HEADDEN, WM. P. 1910 Arsenical poisoning of fruit trees. Colo. Exp. Sta. Bul. 157.

(15) HEADDEN, WM. P. 1924 Some orchard conditions affected by arsenicals, marls, and other factors. Colo. Exp. Sta. Bul. 294: 1–31.

(16) KEHOE, ROBERT A., THAMANN, FREDERICK, AND CHOLAK, JACOB 1933 On the normal absorption and excretion of lead: I. Lead absorption and excretion in primitive life. *Jour. Indus. Hyg.* 15: 257–272.

(17) KEHOE, ROBERT A., THAMANN, FREDERICK, AND CHOLAK, JACOB 1933 On the normal absorption and excretion of lead: II. Lead absorption and lead excretion in modern American life. *Jour. Indus. Hyg.* 15: 273–288.

(18) KEHOE, ROBERT A., THAMANN, FREDERICK, AND CHOLAK, JACOB 1933 On the normal absorption and excretion of lead: III. The sources of normal lead absorption. *Jour. Indus. Hyg.* 15: 290–300.

(19) KEHOE, ROBERT A., THAMANN, FREDERICK, AND CHOLAK, JACOB 1933 On the normal absorption and excretion of lead: IV. Lead absorption and excretion in infants and children. *Jour. Indus. Hyg.* 15: 301–305.

(20) LIPMAN, C. B. 1928 The chemical elements indispensable to plants. *Sci. Mo.* 26: 289–294.

(21) LIPMAN, C. B., AND MACKINNEY, G. 1931 Proof of the essential nature of copper for higher green plants. *Plant Physiol.* 6: 593–599.

(22) McHARGUE, J. S. 1925 The occurrence of copper, manganese, zinc, nickel, and cobalt in soils, plants, and animals and their possible function as vital factors. *Jour. Agr. Res.*, 30: 193–196.

(23) MORRIS, H. E., AND SWINGLE, D. B. 1927 Injury to growing crops caused by the application of arsenical compounds to the soil. *Jour. Agr. Res.* 34: 59–78.

(24) SCHOORL, N. 1909 Beitrage zur Mikrochemische Analyse. Wiesbaden.

(25) SKINNER, W. W. 1906 Copper salts in irrigating waters. *Jour. Amer. Chem. Soc.* 28: 361–368.

(26) SOMMER, A. L. 1931 Copper as an essential for plant growth. Plant Physiol., 6: 339–345.

(27) STEWART, J., AND SMITH, E. S. 1922 Some relations of arsenic to plant growth. *Soil Sci.* 14: 111–126.

(28) VON FELLENBERG, TH. 1930 Über den Arsengehalt natürlicher und mit Arsenpräparaten Behandelter Lebensmittei. *Biochem. Ztschr.* 218: 300–317.

PLATE 1

TYPICAL CRYSTALS OF TRIPLE NITRITE OF POTASSIUM, COPPER, AND LEAD—
$2KNO_2 \cdot Cu(NO_2)_2 \cdot Pb(NO_2)_2$

FIG. 1. From the ash of apples grown in an orchard that never has been sprayed.
FIG. 2. From the ash of pears grown in a commercial orchard.

J. S JONES AND M B HATCH

FIG 1   FIG 2

CHARACTERISTICS OF CERTAIN SOIL PROFILES OF SOUTH-EASTERN WASHINGTON[1]

PAUL I. VLASOFF AND LAWRENCE C. WHEETING[2]

INTRODUCTION

It has been pointed out by several pedologists, particularly in Russia (7, 9, 11), that under different climatic conditions materially unlike soils may be produced from essentially similar rock materials. Appreciable variations in climate occur in different parts of southeastern Washington, and because a relatively uniform soil-forming material covers this region, it should be possible to determine the types of soil development that have resulted from these climatic differences. Consequently, a study was made of both soil and extracted colloidal material in four soil profiles, all derived from similar material but developed under the diverse meteorologic environments of the region

EXPERIMENTAL PLAN

Four soil profiles from localities 25 to 30 miles apart, representing definite rainfall belts along a line extending more than 100 miles from east to west, were selected for this study. The mean annual temperature along this line is fairly uniform and averages about 49°F. January mean temperatures are also uniform and average 28°F. A difference of about 14 inches in mean annual precipitation at the two extremes should be a significant factor in soil development. The mean annual precipitation at the eastern sampling locality is about 20 inches, whereas that at the western extremity is only 6 inches. The region is one in which winter precipitation greatly exceeds the summer rainfall.

Sampling areas were selected where an undisturbed surface layer and the presence of the native vegetation indicated a virgin soil. Samples were collected from five horizons in each profile, representing a depth of 120 inches from top to bottom.

The investigation included studies of:
Morphological characteristics of the profiles in place
Physical characteristics of soils and colloids determined by
  Mechanical analysis
  Moisture equivalent and heat of wetting measurements

Chemical characteristics of soils and colloids determined by
    Reaction and content of carbonates
    Organic carbon and nitrogen
    Silica, sesquioxides, alkaline earth, and alkali bases

MORPHOLOGICAL CHARACTERISTCS OF THE PROFILES

In order that a clear picture of the comparative morphology of the four pro-files may be obtained, the outstanding properties have been brought together in table 1. Attention is called to plates 1 and 2, which should be examined in connection with the tabular material. The distinct character of the lime accu-mulation layer and the formation of streaks directly below it, as shown in the Uniontown profile, are good illustrations of the manner of precipitation of that material in the cracks of root channels, worm holes, and rodent burrows. These streaks of lime often extend 10 feet or more into the parent material. In obtaining samples of this horizon, it was decided that only the more concen-trated lime layer just beneath the solum would be sampled, although this would make a quantitative determination of the total amounts in different profiles impossible. The picture representing a lime accumulation layer in the LaCrosse soil shows plainly one of those tubular hollows and solid inclusions which are simply root passes and crotovinas calcified by the transported lime. The other picture in plate 2 illustrates the distinct prismatic structure of the second and third horizons of the Washtucna soil.

In a recent soil survey of the Columbia Basin Area (17), definite series names were given to certain of these soils. The Uniontown soil is representative of the Palouse series and of Chernozems; the LaCrosse soil is a dark brown soil and may be designated as the Walla Walla series, although in Marbut's con-tribution to the Atlas of American Agriculture (12), there is no distinction made between this soil and the Palouse soil. A morphological study, however, and laboratory analyses which were made of both soils and are presented in this paper, definitely indicate that the LaCrosse soil is much lighter in color than the Palouse soil, which is accounted for by the perceptibly lower organic content. Moreover, the two soils have dissimilar structural characteristics in the lower layers.

The Washtucna soil, according to Marbut's classification, must be placed in the Ritzville series and, as such, designated as a well developed member of the Dark brown soils. A careful consideration of the color of the Washtucna soil, however, which is brown when moist and light brown when dry, suggests placing this soil in the group of Portneuf brown soils. Similarly, the Connell soil, which has been designated by Marbut as Wheeler loam and placed in the group of Brown soils, reveals in reality a light gray color when dry and a brown-ish tinge when moist. It apparently belongs in the gray Serozem group of soils.

The soils described in this paper probably represent four great soil groups of Marbut's category IV: Chernozem, Dark brown, Brown, and Gray.

TABLE 1

Morphological characteristics of the profiles

HORIZON	UNIONTOWN	LACROSSE	WASHTUCNA	CONNELL
1 0–7 in.	Surface soil, black when wet, intensely dark brown when dry, lamellar structure in upper 2 in., changing to faintly granular with depth	Surface soil, dark brown when wet, grayish brown when dry, trace of laminated structure near surface, finely granular throughout	Surface soil, brown when moist, light brown in dry state, lamellar and porous in the upper part, compact in the lower part	Surface soil, light gray when dry, exhibits shades of brown when moist, extremely open and porous
2 7–18 in.	Subsoil, transitory, unevenly dark brown, compact in consistence, slightly reticulate in structure	Subsoil, somewhat light brown, slightly lumpy when dry, perceptibly compact in consistence	Subsoil, light in color with shades of brown, compact, partly columnar, slightly lumpy in structure	Subsoil, transitory gray, decidedly open and porous
3 18–40 in.	Lower subsoil, reddish brown, granular, nutlike in structure, occasional specks of lime in lower portion	Lower subsoil, reddish brown, friable, less compact, finely granular in structure, possesses large number of fine roots of Andropogon	Lower subsoil, grayish brown, loosely granular and distinctly columnar in structure, somewhat porous and open	Lower subsoil, light brown, extremely fine in texture, porous in consistence, gritty and friable
4 40–72 in.	Carbonate layer, grayish brown, abundant with whitish gray lumps or streaks of lime, somewhat sandy in texture, effervesces in acid	Carbonate layer, light brown with white lumps and tubular hollows of lime, friable and sandy in texture, effervesces in acid	Carbonate layer, whitish gray, abundant with large lumps and streaks of lime, open in constitution, effervesces violently with acid	Carbonate layer, yellowish gray in color, specks and granules of lime, extremely porous and gritty, effervesces in acid
5 72–120 in.	Parent material, free of lime, brownish gray, mellow, open and sandy, loess-like	Parent material, free of lime, mellow, yellowish gray, open and sandy, loess-like	Parent material, free of lime, gray, loose-grained, mellow, porous and sandy, loess-like	Parent material, free of lime, gray, finely aggregated with occasional large lumps, sandy in texture, resembles loess
Annual rainfall	*20 in.*	*15 in.*	*12 in.*	*6 in.*

PHYSICAL STUDIES OF SOILS AND COLLOIDS

Mechanical analysis

Mechanical analysis was made of each individual horizon, using the Bouy-oucos (3) hydrometer method. The results given in table 2 make it appear that the clay content of each profile follows some pedologic order; that is, it increases from horizon 1 to 2, where it is at a maximum, and then decreases in the lower horizons. The clay content of the corresponding

TABLE 2

Mechanical analysis of soils from which the organic matter and carbonates were removed

SOIL	HORI-ZON	DEPTH	MEDIUM SAND	FINE SAND	VERY FINE SAND	SILT	CLAY	CLASS
		inches	per cent	per cent	per cent	per cent	per cent	
Uniontown	1	0–7	0.1	1 3	27.0	40 0	31.6	clay
	2	7–18	0.1	0.8	27.5	36.0	35.6	clay
	3	18–40	0.1	0 4	29.1	40.8	29.6	clay loam
	4	40–72	0.2	1 5	36.3	40.4	21.6	clay loam
	5	72–120	0 0	0.2	39.7	44.8	15.2	loam
LaCrosse	1	0–7	0.1	1.2	33.8	40.4	24.4	clay loam
	2	7–18	0.1	1.1	30.3	41.2	27.2	clay loam
	3	18–40	0.1	1 0	30.3	48.0	20.4	clay loam
	4	40–72	0.1	1.7	33.1	46.4	18.4	loam
	5	72–120	0.1	0.5	44.1	43.6	11.2	loam
Washtucna	1	0–7	0.2	1.7	32.3	46.0	19.6	loam
	2	7–18	0.1	1.2	37.6	40.4	20 4	clay loam
	3	18–40	0.3	2.5	38.7	40.4	18 0	loam
	4	40–72	0.1	1.2	39 9	44.4	14 0	loam
	5	72–120	0.0	0 2	42.6	48.0	8.2	loam
Connell	1	0–7	0.3	2 3	42.9	44.4	10.0	loam
	2	7–18	0.6	5.2	37.9	44.0	12.4	loam
	3	18–40	1 0	8.7	39.7	40.4	11.2	loam
	4	40–72	0 9	7.3	44.5	36.4	11.2	loam
	5	72–120	0.0	0.1	40.7	49.8	9.4	loam

weathered horizons of the four profiles decreases as the rainfall decreases, but in horizon 5, the unweathered layer, no significant variation occurs. The higher value for clay in horizon 5 of the Uniontown soil probably is within the experimental error of the method since the combined amounts of silt and clay in all corresponding horizons are comparable.

The sand fraction of each horizon falls entirely into three size groups: medium sand, fine sand, and very fine sand. The sand fraction from horizon 5 of all four profiles is almost all very fine sand, and since the silt clay and clay fractions did not vary significantly, it is apparent that the texture of the parent

material is essentially similar. The possibility that it is wind laid is also suggested.

According to the mechanical analyses and textural classifications proposed by the Bureau of Chemistry and Soils, three soils classes may be distinguished, as is shown in table 2. The range in texture of the surface soils is from a clay on the east in the region of greater rainfall to a loam on the west. Since the parent material is similar, the climatic factor of moisture is definitely effective in the development of widely different textural characteristics within the solum of the different profiles.

TABLE 3

Moisture equivalent of the soils

SOIL	HORIZON	DEPTH	MOISTURE EQUIVALENT
		inches	*per cent*
Uniontown	1	0-7	27.9
	2	7-18	26 1
	3	18-40	25 5
	4	42-72	24 9
	5	72-120	16 8
LaCrosse	1	0-7	23.8
	2	7-18	21.6
	3	18-40	18 9
	4	40-72	21.4
	5	72-120	14 6
Washtucna	1	0-7	20 3
	2	7-18	18 4
	3	18-40	17 8
	4	40-72	21 8
	5	72-120	14 7
Connell	1	0-7	16.3
	2	7-18	15.9
	3	18-40	15.7
	4	40-72	17.3
	5	72-120	13.7

Moisture equivalent

Values for soil moisture equivalents were obtained by using the Briggs and McLane (5) centrifugal machine. Corresponding horizons of the profiles from the low rainfall belts hold smaller amounts of water than those from the high rainfall sections. This is associated with two variables which follow the same trend: the amount of fine mineral material and the amount of organic matter. The slight increase in the moisture equivalent values in the carbonate horizons of the soils from the dryer districts as compared with those of their overlying horizons must be associated with the large amounts of internal sur-

face resulting from the colloidal character of the carbonate deposits, as data reported later on heat of wetting show that the intensity of the water retention is actually lower in layers containing carbonates. Thus, the greater total retaining capacity must come largely from increased surface area within these horizons. Under the higher rainfall conditions, soils have developed which are capable of holding large amounts of moisture. The more xerophytic conditions in the western part of the area have resulted in the formation of soils with smaller water-holding capacities.

TABLE 4

Heats of wetting and percentage of colloid calculated from them

SOIL	HORIZON	DEPTH	H.W. CAL./GM. SOIL	H.W. CAL../GM COLLOID	COLLOID
		inches			*per cent*
Uniontown	1	0–7	4 304	11 821	36 409
	2	7–18	4 696	11.178	42 011
	3	18–40	4 625	10 691	43 260
	4	40–72	3 178	7.091	44 817
	5	72–120	1 396	6 300	22.160
LaCrosse	1	0–7	2 743	9.685	28 323
	2	7–18	3 497	9 511	36 767
	3	18–40	2.775	9 498	29.216
	4	40–72	1 947	5 879	33.117
	5	72–120	1 108	6 000	18.460
Washtucna	1	0–7	2 169	10 070	21.539
	2	7–18	2 330	9.584	24.311
	3	18–40	2.171	9 575	22.673
	4	40–72	1.148	2 855	40.210
	5	72–120	1 048	6 080	17.230
Connell	1	0–7	1.903	8 846	21.501
	2	7–18	1 896	8.315	22.802
	3	18–40	2.556	8.333	30.673
	4	40–72	2.750	7.930	34.673
	5	72–120	1.048	6 080	17.260

Heat of wetting

The determination of the heat of wetting of whole soils and of colloids, which give a measure of the energy evolved in the water absorption process, can be readily obtained, and the attractive power of different soils for water can be calculated in energy units. The Bouyoucos (2) experiments pertaining to this subject indicate that coarse sandy soils are practically free of colloids and therefore give no heat of wetting, whereas colloidal clay may give more than 1,200 calories per 100 gm. of soil.

The colloidal portion of the soils was obtained by a method similar to that used by Bradfield (4), except that the original dispersion was done with a

Bouyoucos stirrer, and the time of settling was reduced so that particles as large as 5μ in diameter were included. One separation was made with the Sharples supercentrifuge, which gave coarse colloidal material, including particles from 5μ to 0.3μ in diameter and fine colloidal material with particles smaller than 0.3μ in diameter. The finer fraction was collected by using the centrifuge as suggested by Bradfield, since he found that only a small quantity of colloidal material is not recovered by this method. The material was air-dried, ground to a fine powder, and stored for experimental use. For heat of wetting studies, the entire colloidal fraction, including particles with diameters as large as 5μ, was used. For chemical studies, only the finer fraction, with particles smaller than 0.3μ, was used.

By using the method of Bouyoucos (1) for heat of wetting, the results presented in table 4 were obtained. Considering the data for soils first, it may be observed that the highest figure was obtained with the Uniontown soil, which is the highest in humus and sesquioxides. The decided drop in the heat of wetting occurring in horizon 4 of all profiles is undoubtedly due to the large quantities of carbonates, which result in a comparative reduction in the amount of colloidal constituents such as SiO_2, Al_2O_3, Fe_2O_3, and humus, as will be shown later. That a low heat of wetting is obtained when a high percentage of carbonates is present is also suggested by Bouyoucos (2). The figures for the parent materials in three profiles were practically the same except that the Uniontown soil yielded a slightly higher value.

The percentage of colloids calculated by the ratio suggested by Bouyoucos is not in agreement with the clay content according to the data obtained by mechanical analysis. Bouyoucos accounts for this by assuming that some of the silt particles as determined by mechanical analysis are in reality colloidal aggregates. If this assumption is valid, the quantities of colloidal material as determined here by heat of wetting measurements might be expected to exceed the quantities of clay as determined in mechanical analysis.

CHEMICAL STUDIES OF SOILS AND COLLOIDS

Reaction and carbonates

The reaction was determined by means of the quinhydrone electrode, using the ratio of soil to water of 1:4, and the carbonates by means of Scheibler's calcimeter. The data are shown in table 5. It was found that the pH values of the soils, as taken from the field, range from 6.82 in horizon 3 of the Uniontown soil to 8.98 in horizon 4 of the Connell soil. The latter value and certain others above pH 8.4 are subject to some question because of the limitations of the quinhydrone method. Generally, the corresponding soil horizons were more alkaline in the more arid regions than in the humid section. In all four profiles, either horizon 2 or 3 was less alkaline than the surface horizon, and in horizon 3 of the Uniontown profile, an acid reaction was found.

The reaction of the extracted colloidal material was in no way related to the reaction of the original soil. The most acid colloidal material gave a pH value

of 6.10 and was derived from the surface horizon of the Uniontown profile. All of the other colloids were alkaline in reaction and gave evidence of base saturation.

It was interesting to find such enormous quantities of carbonates in the fourth horizon of all of the profiles and, especially, in the colloids extracted from these horizons. Judging from the high $CaCO_3$ content of the colloids in the fourth horizon of each of these profiles, the assumption may be made that horizon 4 is essentially an illuvial lime accumulation layer. It is believed

TABLE 5

Reaction and carbonates of soils and colloids

SOIL	HORIZON	REACTION		CARBONATES	
		Soil	Colloid	Soil	Colloid
		pH	*pH*	*per cent*	*per cent*
Uniontown	1	7.18	6 10	0 000	0 000
	2	7 03	7 29	0 000	0 000
	3	6 82	7 83	trace	trace
	4	8 53	8 04	8 772	21 954
	5	8 02	8 38	0 000	0 000
LaCrosse	1	7 12	7 53	0 000	0 000
	2	7 03	7.87	0 000	0 000
	3	7 87	7 78	trace	trace
	4	8 81	8.38	9 138	25 979
	5	8.64	8 64	0 000	0 000
Washtucna	1	7.70	7.78	0 000	0 000
	2	7.20	7 37	0 000	0 000
	3	7 46	7 35	trace	trace
	4	8 64	8 21	14 986	39 883
	5	8 78	8.89	0 000	0 000
Connell	1	8.13	7 79	0.000	0 000
	2	7.70	7 53	0 000	0 000
	3	7 96	7 37	trace	trace
	4	8.98	8 38	3.655	10 977
	5	8.89	8.78	0 000	0.000

that the calcium bicarbonate formed as a result of the decay of organic matter in the surface horizons has been transported downward in periods of heavy rainfall to the point of maximum water penetration. During the following dry season, this material was precipitated in place, much of it in the form of colloidal calcium carbonate. Since no indication of the presence of calcium carbonate was found in the parent material, the accumulation in the fourth horizon is obviously the result of the activity of plants and microorganisms and climatic agencies.

The depth at which carbonate accumulation began was nearly the same for

the profiles studied, regardless of total annual rainfall. It should be understood, however, that in each soil zone carbonates are formed at variable depths. The uniform depth at which the carbonate layers lie in the sampled profiles may possibly be accounted for by considering the penetration of the winter rainfall. Although the total rainfall is greater at the eastern sampling point, evidently no greater penetration is secured there, if the depth of the carbonate layer can be used as an indication of average moisture penetration. This may be due to the fact that not only must the moisture received in the winter saturate a much heavier textured soil, but, also, greater quantities of it are lost in runoff. The decrease in carbonate accumulation in the Connell soil is perhaps associated with the almost complete substitution of a native vegetation of sage brush (*Artemesia tridentata*) for the grass vegetation of the other three soils.

Organic matter, carbon, and nitrogen

The outstanding importance of these soil constituents has been recognized for a long time and recently several pedologists (10, 14, 18) have strongly emphasized the rôle of organic life in soil formation.

Analyses for carbon were made on both the whole soils and the colloidal fractions by means of the Schollenberger (16) method, modified somewhat in this laboratory. Data obtained for carbon were converted to organic matter by multiplying by the usual factor, 1.724.

The results of the determinations for organic matter as they are expressed in table 6 indicate that there is a gradual decrease of this constituent with depth in the profiles, both in the whole soil and in the colloidal fractions.

As to the relative amounts of organic matter in different profiles, the first two horizons of each profile have been very strongly affected by the decrease in precipitation, as is indicated by the significant diminishing in organic matter content noticed upon proceeding in a westerly direction across the area. From horizon 3 downward, there were no great variations in the quantity of organic matter, indicating that this must be the depth to which maximum plant root activity is limited regardless of annual precipitation. The occurrence of more than 1 per cent of organic matter in the lower layers of the soil and even in the parent material itself may indicate that the parent material was of loessial origin, because it would have been possible for organic substances to have become uniformly mixed with mineral matter as a result of wind deposition. The colloidal fractions extracted from the different horizons are in general richer in organic matter than the corresponding soil, especially in the case of the surface layers. A greater proportion of the soil organic matter is evidently of colloidal character.

Nitrogen, as determined by the Kjeldahl method, follows the same trend as organic matter, showing a diminution with depth in each profile and a progressive diminution as the arid zone is approached.

The carbon-nitrogen ratios in two soils vary greatly, a wider ratio being

associated with the region of low rainfall.   The ratios obtained with extracted colloidal material, however, are remarkably uniform, if the values for horizon 4 in each profile are disregarded.   The abnormal values in this horizon are the result of the carbonate accumulations.   The uniformity of the ratios suggests the conclusion that throughout this region the colloidal organic matter derived from organic decomposition processes approaches a constant composition value with respect to carbon and nitrogen, at which it remains relatively stable.

TABLE 6

Organic matter, nitrogen and carbon, and C:N ratio of soils and colloids

SOIL	HORI-ZON	ORGANIC MATTER		NITROGEN		CARBON		C.N	
		Soil	Colloid	Soil	Colloid	Soil	Colloid	Soil	Colloid
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
Uniontown	1	4.215	6.254	0.183	0 430	2 450	3.615	13	8
	2	2.579	4.053	0 086	0 305	1.491	2 343	17	8
	3	1.468	1.759	0.027	0.106	0.849	1 017	31	10
	4	1 079	1.401	0.026	0.111	0.624	0.486	24	4
	5	1 349	1.323	0.014	0.089	0.780	0.765	56	9
LaCrosse	1	2.963	5 304	.....	0 429	.....	3 066	..	7
	2	2.402	5.029	... .	0.368	.....	2 907	.	8
	3	1.598	2.699	.....	0.240	.....	1.560	.	7
	4	1.168	1.084	. ...	0.127	.....	0.627	.	5
	5	....	1.294	. . .	0.068	.....	0.690	..	8
Washtucna	1	2.470	5.486	.....	0.412	.....	2.955	..	7
	2	1.884	4.878	. ...	0.373	...	2.820	..	7
	3	1.775	4.180	....	0.364	.....	2.415	..	7
	4	1.287	1.577	. .	0.111	.....	0.515	.	4
	5		1.557	.....	0.109	. ..	0.900	.	8
Connell	1	1.583	3 866	0.053	0.285	0.915	2.235	16	8
	2	1.474	2.942	0.038	0.246	0.852	1.701	22	7
	3	1.313	2.776	0.032	0.235	0.759	1.605	23	7
	4	1.105	1.738	0.018	0.146	0.639	0.600	35	4
	5	1 349	1.477	0 014	0.105	0.780	0.900	55	9

Silica and sesquioxides

The inorganic constituents of all of the soil colloids and of two soils were analyzed, by fusing the soil material with Na_2CO_3 and then determining the separate ingredients in the hydrochloric acid extract of the fused material, using the official A.O.A.C. methods. Table 7 gives the results for silica and the sesquioxides.   The importance of the colloidal alumino-silicate compounds in soil development processes may be emphasized, since these colloidal compounds appear to be the best indicators of the degree of rock weathering that has taken place in the different profiles.

The analytical results show a remarkably uniform composition for the col-

loidal fraction over the entire range of annual rainfall within the area studied. The values for the silica sesquioxide ratio lie between 3.0 and 3.88 in all cases but one. A difference in average annual rainfall of 14 inches has but little influence on these ratios.

The average composition of the colloidal matter for this district is like that of soil colloids in the Great Plains, although they are richer in silica and poorer in alumina than the pedalfer colloids of the humid section of the United States.

TABLE 7

Silica and sesquioxides of colloids and soils

SOIL	HORI-ZON	COLLOID				SOIL		
		$\dfrac{SiO_2}{Al_2O_3 + Fe_2O_3}$	SiO$_2$	Al$_2$O$_3$	Fe$_2$O$_3$	SiO$_2$	Al$_2$O$_3$	Fe$_2$O$_3$
		gm -mols	per cent	per cent	per cent	per cent	per cent	per cent
Uniontown	1	3 04	54.012	24.610	8.493	69.166	18.915	5.396
	2	2 90	52.606	27.823	8.888	69 149	19.465	4.785
	3	3 06	55.020	25.301	8.244	69.914	18 938	5.315
	4	3 39	41.948	18 050	4 690	71.940	17.167	4.608
	5	3.10	55.458	24.868	8.765	70.295	18.354	4.524
LaCrosse	1	3 88	58.263	22.070	7.282	.. ..	. ....	. ....
	2	3.28	54.554	22.992	8.163	. ....	......	.. ..
	3	3.17	55.079	24.395	8.036	. ....	.. ...	. ....
	4	3.05	38.689	17.887	5.726	. . ...	. ....	. ...
	5	3 11	54.511	24.822	8.245	. ....	. ....	. ...
Washtucna	1	3.74	58.269	21.241	8.135	... ..	......	. ....
	2	3.38	57.071	23.625	8.212	......	......	. ....
	3	3.14	56 972	25.316	8.654	. ....	......	... .
	4	3.24	31.900	13 683	4.849	..... .	.. ...	. ....
	5	3 14	53.663	24 020	8 653	.. ..	......	. ....
Connell	1	3 66	58.590	21 944	8.134	69.439	16.432	4.795
	2	3.69	57.915	22.381	8.653	68.654	17.097	5.594
	3	3 55	57.781	22.983	7.616	68.174	17.644	6 321
	4	3.46	47 681	19 004	6.857	67.148	17.771	5 340
	5	3 00	52.355	24.531	8.871	73.430	17.149	4.182

Brown and Byers (6) report an average silica sesquioxide ratio of 3.31 for 13 dry land profiles in the Great Plains area. The average value for this ratio in the four profiles in southeastern Washington is 3.30, values for the individual profiles from east to west being 3.10, 3.30, 3.33, and 3.47. The pyrophyllite type of compound, as suggested by Brown and Byers, is no doubt representative of the colloidal fraction of the southeastern Washington soils.

Alkaline earth and alkali bases

Calcium and Magnesium. These constituents were determined from the solutions of the sodium carbonate fusion after removal of silica and sesquioxides

in extracted colloids and on two soils, using the official A.O.A.C. methods, with minor modifications. The results obtained appear in table 8. Decreasing amounts of calcium were found in passing from the first to the third horizon in each profile. The perceptibly accumulated quantities of calcium in the surface layer apparently have been brought into this horizon by virtue of the action of a grass vegetation. The latter, somehow, being limited in the development of an extensive root system, may have been obliged to obtain its calcium

TABLE 8

Total bases in colloids and soils

SOIL	HORI-ZON	COLLOID		SOIL		COLLOID		SOIL	
		Ca	Mg	Ca	Mg	Na	K	Na	K
		per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Uniontown	1	1.745	1.607	2.700	1.444	2.987	3 562	3.026	2 377
	2	1 322	1 734	1.541	1.252	2.736	2 702	3 020	2.399
	3	1.237	2 053	1.503	1.522	2.757	2.415	2.791	2.100
	4	10.549	1.911	5.212	1.478	2 963	1.708	2.664	2 038
	5	1.580	2.843	1.787	1.220	2 695	2 484	3 283	2 324
LaCrosse	1	1.614	1.489	.....	....	2 916	2 530	. .	. . .
	2	1.305	1.729	.....	.. .	2.615	2 311	.	...
	3	1.108	2.098	.....	. .	2.726	2.605	. ...	.. .
	4	12 068	1.976	.. .	. . .	2.678	1.678	. . .	...
	5	1.354	2.979	. ...	.....	2.877	2.381	..	. ..
Washtucna	1	1.614	1.628	.....	. .	2.449	2.471	. . .	. . .
	2	1.584	1.768	. . ..	.. .	2 850	1 974	. . .	.. .
	3	1.603	2 206	. . .	....	2.838	1.972	. . .	....
	4	16.257	2 027	.. ..	....	2 576	2.121	.	..
	5	1.463	2.129	. . ..	.	2 667	2 446	. .	. .. .
Connell	1	1.605	1 923	2.306	1.786	2.305	2 315	3.434	2.270
	2	1.568	1.943	2.059	1.600	2.604	2.421	3.019	2.337
	3	1.514	2 555	2.281	1.928	2.416	1.464	3.279	2.111
	4	5.911	2.173	2.341	1.751	2.540	1.986	2.283	2 072
	5	1 724	2.827	1.810	1.292	2.826	2 511	3.379	2 212

supply largely from subhorizons 2 and 3, consequently impoverishing the latter of available calcium compounds. The somewhat unusually large amounts of calcium present in horizon 4 can be explained by assuming that the soils have been undergoing the process of "pedocal" formation, which permits the production of soluble $CaHCO_3$ in the presence of sufficient amounts of decaying organic matter. The calcium bicarbonate thus formed in the upper layers has been translocated to greater depths in periods of abundance of moisture, as previously explained. Data for calcium for the parent material horizon seem fully in agreement in all profiles, and perhaps indicate that

although horizon 5 immediately adjoins the carbonate layer, it has not been influenced by soil-forming processes.

The calcium data for soil are analogous to those for colloids and differ only in the amounts of this constituent. The greater quantities were obtained in the whole soils, except in the case of horizon 4 of each profile. This indicates that the accumulated $CaCO_3$ of horizon 4 in each case was largely precipitated as colloidal material, whereas the calcium normally present in the soil is in a non-colloidal form.

Tracing the data for magnesium and comparing it with the results for calcium, both as constituents of the colloidal fractions, it may be inferred that the processes of accumulation of these two elements are not alike. Within the solum, the largest quantities of calcium appear in horizon 1, whereas the accumulation of magnesium compounds takes place mostly in the third horizon, which might indicate that the intensity of its transport by plants to the surface is considerably less than is the case with calcium. Also, the assumption that magnesium forms a more stable combination with the silicate group and is therefore displaced with greater difficulty might explain why magnesium was not in an available form to the plants for translocation to the surface. If this is true, the leaching of magnesium cannot be effected to any appreciable extent; therefore, the relatively high figures for magnesium in horizon 3 suggest that much more of this constituent is retained by the colloidal fractions than by the undispersed whole soils. That the soil colloids of the semi-arid regions are relatively richer in magnesium than those in more humid regions is suggested by Mattson (13). Horizons 4 and 5 seem to show that the magnesium content of the colloids of these layers has not been altered to any extent by soil formation processes, as indicated by the uniformity of the data for magnesium. That a low figure for magnesium was obtained in the carbonate layer evidently stresses the fact that the removal of carbonate material from the surface soil occurred predominantly in form of $CaHCO_3$.

Concerning the magnesium data for whole soils, it must be stated that considerably smaller figures for this constituent were obtained in soils, as compared to the colloids from comparable horizons. This indicates a strong tendency for magnesium to combine with the alumino-silicate colloidal complex. It is of interest to note, however, that in the parent material slightly less than half of the total magnesium present is contained in the colloidal fraction from that horizon. Since the material has not been weathered appreciably, this would indicate that the fine particles of the soils contained more magnesium originally than the coarse particles. This will not account entirely, however, for the differences observed in the weathered horizons.

Sodium and Potassium. For the purpose of analysis of these two constituents, separate fusions by means of the J. Lawrence Smith method were made. The actual determinations of sodium and potassium on the aliquots prepared after fusion were made by the uranyl-zinc-acetate gravimetric method and by the sodium-cobalti-nitrite volumetric method, respectively. The values

secured for whole soils and colloids are also presented in table 8.   It should be pointed out that the sodium content of the colloids and that of the whole soils varies only a small fraction of a per cent, and the larger amounts, as a rule, appear in the whole soils, excluding the carbonate layers, where these figures are higher in the case of the colloids.   The higher figure for sodium in the colloid from the carbonate layer of the Uniontown profile indicates that soluble sodium has been produced through weathering of the minerals in the upper horizons, and that these soluble sodium compounds accompanied the lime salts downward and were precipitated in the fourth horizon upon desiccation.

The data for potassium in several respects show the same trends as those for magnesium.   The potassium of the soils appears to be a constituent of only slightly soluble compounds and, therefore, is rendered relatively immobile, similarly to the magnesium compounds.   The evidence of accumulation of the potassium in the surface horizon and the diminution of it in the two lower horizons is, however, not demonstrated by the magnesium, and this possibly suggests that the former has been conveyed more freely to the surface, where it became fixed in the form of slightly dissociated compounds and was thus preserved from leaching.   The greater quantities of potassium shown by the colloidal data, as compared to the figures for the whole soils, is further proof of the probable organic combination of potassium, because the colloids have been previously shown to be richer in the organic constituents.

GENERAL DISCUSSION

From a consideration of the data presented, it would appear to be necessary to study the composition of the soil colloids rather than the whole soil in order to understand the nature of soil formation processes.   This is especially true in connection with the analyses of silica and sesquioxides, alkaline earth and alkaline bases, and organic matter.

The results of the field and laboratory studies of the four profiles investigated indicate that they represent pedocal soils.   The pedocal process of soil formation usually assumes the presence of a lime accumulation layer at some depths in the solum, originated by the percolation of calcareous material from the surface soil and the deposition of it in a lower layer.   Lime accumulation layers were found in each profile, yet carbonates were not present in the parent material, which points out a peculiarity of these soils in comparison with certain other types of pedocal profiles, such as the Barnes soils of the Great Plains.   Since there is no indication of any other source of lime being conveyed into horizon 4, the large amounts of the $CaCO_3$ present in this layer may be looked upon as the illuvial accumulated material transported from the surface soil and deposited by desiccation and precipitation during certain seasons of the year.

Another point of interest in connection with these soil profiles is the organic matter.   Examinations showed that there is a decrease in the amount of organic matter from east to west and also with depth in the profiles.   There is a

rather conclusive indication that the diminution in organic matter content is due to decreasing density of plant associations toward the arid zone, which in turn is conditioned by the descending gradient in precipitation from east to west.

The findings of several investigators, including Hilgard (8) and Remezov (15), in relation to the nitrogen content of the organic matter under different rainfall conditions are not valid for these soils which show no direct relation between the precipitation and the nitrogen content. The soils receiving about 6 inches of rain per year contain approximately the same percentages of nitrogen in their organic matter as those receiving in the neighborhood of 20 inches per annum, and the nitrogen-carbon ratios, especially for the colloids, are fairly constant.

The Uniontown soil, although placed in the group of Chernozem soils, appears to have much in common with the Chestnut soils of the Stavropol district, Russia, as described by Zakharov (18), who found that they contain about 3 per cent of organic matter in the surface horizons and steadily decreasing amounts with increasing depth. Similar quantities of organic matter in the surface layers and similarly diminishing amounts in the lower horizons were found in the soils under consideration here.

Nowhere in the entire region covered by Palouse soils, to which the Uniontown soil belongs, are the amounts of organic matter appreciably larger, as is shown by the extensive studies by workers at the Washington Agricultural Experiment Station. The average quantities of organic matter reported by these workers for 51 samples was 3.24 per cent. The establishment of a definite percentage for organic matter as a boundary between Chernozem and Chestnut soils is probably not feasible without more investigation.

The textural characteristics of the different soil profiles proved to be of considerable interest. It is obvious that the sandy character of the solum becomes more pronounced as one approaches the more arid regions, and correspondingly, the clay material diminishes as one proceeds from east to west. The silt fraction, however, appeared to be almost a constant percentage throughout the area investigated. That the latter remained uniform in amounts, may indicate that a somewhat similar physical disintegration of the mineral part of the soil is common to the four profiles. The size distribution of sand particles, being similar in all profiles, is further proof of the identity of the physical soil-forming processes. The information concerning the variability of texture with depth shows that the second horizon is outstanding in all profiles for its high clay content, when compared to the clay content of lower or higher layers. This may be the result of a slight eluviation from horizon 1.

Studies of the soil reaction, especially of the colloids, show that a somewhat basic reaction predominates in all horizons except the surface layer of the Uniontown profile, which exhibits a weakly acidic reaction. Perhaps this also indicates some eluviation activities in the upper part of this profile. It may be

concluded that no significant translocation of sesquioxides will occur as long as the existing pH values persist.

The thickness of a profile is of significance in drawing conclusions regarding the extent of weathering of the different soils. Approximately the same thickness of solum was found throughout the area of sampling. This indicates that the total annual rainfall is not as important as the depth of penetration during the season of heavy rains.

The weak leaching and slight eluviation are largely responsible for the conservation of the mineral and organic constituents in their original positions and result in the development of remarkably uniform profile characteristics, even under considerable variation in rainfall.

The uniformity of the silica content and of sesquioxides and other bases suggests that under present conditions of soil reaction and of the concentration of moisture mainly in the surface horizons, conditions are not favorable for strong changes in composition. Consequently, the $\dfrac{SiO_2}{Al_2O_3 + Fe_2O_3}$ ratio is shown varying only within the limits of 3.00 to 3.88.

The results obtained would suggest the following nomenclature for the individual horizons of the four profiles investigated:

Horizon 1 may be designated as A_1
Horizon 2 may be designated as A_2
Horizon 3 may be designated as B
Horizon 4 may be designated as C_1
Horizon 5 may be designated as C_2

A complication arises in connection with horizon 4 because there is a choice of naming it either B_2 or C_1. Because of the presence of illuvial carbonate material, it should be called B_2. But upon considering the similarity in the composition of horizons 4 and 5, excluding the carbonates from horizon 4, it would seem more descriptive to designate this horizon C_1, because it is not soil material but has simply been the recipient of illuvial carbonates. This would appear to be a workable designation for the horizons of these and similar types of Pedocal soils.

SUMMARY

Four soil profiles in southeastern Washington, developed under different conditions of precipitation, were studied.

The data show that the texture of the soils becomes coarser from east to west because of differences in rainfall during the process of weathering.

The water-holding capacity of the soils increases with a corresponding increase in the colloidal fraction or in the organic fraction.

The heat of wetting of soil colloids was found to be much higher than the heat of wetting of soils. The heat of wetting of soil generally decreases from east to west when corresponding horizons are considered.

The nitrogen content follows the trend of the organic matter content; it decreases in crossing the area from east to west.   The quality of the colloidal organic matter of the arid and semi-arid soils appears to be the same.

The reaction of the whole soils and colloids was found to be generally alkaline.   No sharp variations were noticed, except in the Uniontown surface soil colloid, which had a slightly acid reaction.

The results of the chemical analyses indicate that silica and sesquioxides were present in fairly uniform amounts in all horizons, with the exception of the carbonate layer of each profile and of horizon 2 of the Uniontown soil. These deviations have been explained as the consequence of the translocation processes functioning in the soil.

The $\dfrac{SiO_2}{Al_2O_4 + Fe_2O_3}$ ratio for extracted colloids varies within the limits of 3.00 to 3.88 throughout the four profiles, which indicates that the weathering processes producing these materials have been essentially similar.

The percentage of calcium and of magnesium in the soil colloids from the upper three horizons changes with increasing depth in the solum, the calcium content decreasing, and the magnesium content increasing.   Calcium appears to be a constituent of the coarser fractions; magnesium of the finer fractions. An illuvial lime accumulation layer is a feature of all profiles.

The variations in sodium content of the extracted soil colloids were so small that no significant deduction can be made from them.

From a consideration of the data presented, it was concluded that all of the profiles studied represent examples of pedocal formation.   Although but one representative of each zonal group of soils was analyzed, the results indicate differences which would be significant in making a classification.   A nomenclature for horizons of such soils is suggested.

REFERENCES

(1) BOUYOUCOS, G. J. 1925 Heat of wetting, a new means of estimating the colloidal material in soils. *Soil Sci.* 19: 153–162.

(2) BOUYOUCOS, G. J. 1925 The chief factors which influence the heat of wetting of soil colloids. *Soil Sci.* 19: 477–482.

(3) BOUYOUCOS, G. J. 1926 The hydrometer as a new method for the mechanical analysis of soils. *Soil Sci.* 23: 343–353.

(4) BRADFIELD, R. 1923 The chemical nature of colloidal clay. Mo. Agr. Exp. Sta. Res. Bul. 60.

(5) BRIGGS, L. J., AND MCLANE, J. W. 1907 The moisture equivalent of soils. U. S. Dept. Agr. Bur. Soils Bul. 45.

(6) BROWN, IRVIN C., AND BYERS, HORACE G. 1935 The chemical and physical properties of dry land soils and of their colloids. U. S. Dept. Agr Tech. Bul. 502.

(7) GLINKA, K. A. 1927 Pochvovedenie. [Russian.] Moscow and Leningrad.

(8) HILGARD, E. W. 1892 Relation of soil to climate. U. S. Dept. Agr. Weather Bur. Bul. 3.

(9) HILGARD, E. W. 1921 Soils. The Macmillan Co., New York.

(10) KELLOGG, C. E. 1936 Development and significance of the great soil groups of the United States. U. S. Dept. Agr. Misc. Pub. 229.

(11) KOSTYCHEV, P.　1886　The Soils of the Chernozem Region of Russia, vol. 1 [Russian]. St. Petersburg.
(12) MARBUT, C. F.　1935　Atlas of American Agriculture, Pt. III.
(13) MATTSON, SANTE, AND HESTER, J. B.　1933　The laws of soil colloidal behavior.　XI: Electrodialysis in relation to soil processes.　*Soil Sci.* 36: 149–163.
(14) NIKIFOROFF, C. C.　1936　Weathering and soil formation.　*Third Internatl. Cong. Soil Sci.* 1935 1: 324–326.
(15) REMEZOV, N. P.　1933　On the qualitative composition of the organic substances of soils of the U. S. R. R.　*Pedologie* 5: 383–395. [Russian].
(16) SCHOLLENBERGER, C. J.　1930　Determination of soil organic matter.　*Soil Sci.* 31: 483–486.
(17) STRAHORN, A. T., ET AL.　1929　Soil survey of Columbia Basin area, Washington. No. 28, Bur. Chem. and Soils, U. S. Dept. Agr.
(18) ZAKHAROV, C. A.　1933　A Course in Soils.　[Russian].　Leningrad.

PLATE 1

THE UNIONTOWN SOIL PROFILE

Stick 7 meters long

PLATE 1

PAUL I. VLASOFF AND LAWRENCE C. WHEELING
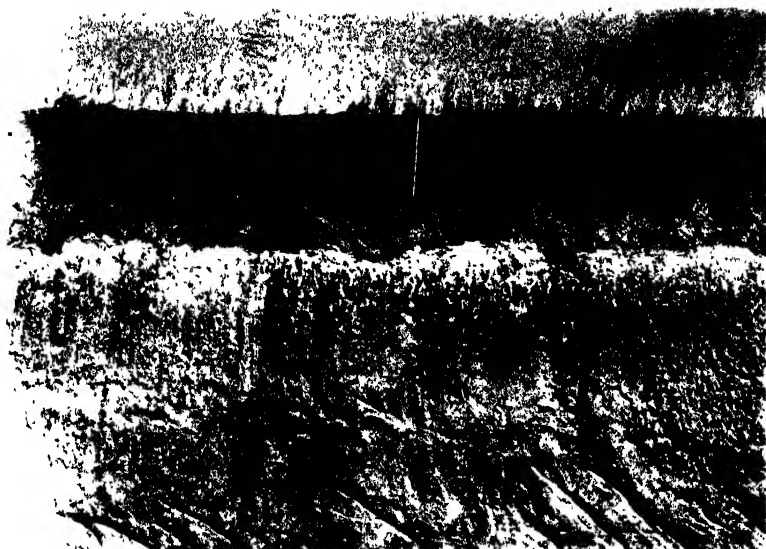
PLATE 2

FIG. 1. LIME CONCRETIONS OF THE LaCROSSE SOIL PROFILE
FIG. 2 COLUMNAR STRUCTURE OF THE WASHTUCNA PROFILE

,

PAUL I VLASOFF AND LAWRENCE C WHEETING

FIG 1

FIG. 2

THE DESTRUCTION OF ORGANIC MATTER IN THE PRELIMINARY TREATMENT OF SOILS FOR MECHANICAL ANALYSIS

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The oxidation of organic matter before dispersing soils for mechanical analysis is a recognized procedure in most laboratories. The present paper deals only with the problem of to what extent the different methods of oxidation succeed in destroying the organic matter in soils. The three standard procedures so far developed are:

(*a*) Oxidation with H_2O_2 (2)
(*b*) Oxidation with hypobromite (3)
(*c*) Oxidation with alkaline $KMnO_4$ (1)

Humic matter was determined by three methods, as outlined below, after the soil organic matter was destroyed by procedures (*a*), (*b*), and (*c*).

(*A*) Colorimetrically: by shaking 5 gm. of the soil with a 100 cc. mixture of 0.5 N NaOH and 0.2 N Na_2CO_3, followed by filtration after the addition of NaCl to flocculate the soil. The color of the filtrate was compared with standard solutions of Merck's humic acid. The reliability of this method may be questioned, but we shall discuss this point in another publication. For the present purpose, when we are interested in comparative values before and after a specified treatment, it is possible that there is no objection to such a procedure.

(*B*) Walkley and Black's method (4): titration with $FeSO_4$ in the presence of chromic acid.

(*C*) Loss on ignition: Estimation of organic matter by loss on ignition is considered to be an extremely unreliable method. It was included in this study, however, because many workers take the ignited weight of the various fractions separated in the mechanical analysis, and a comparative statement as to how the ignited weights are likely to be affected would be of interest.

Nine soils were used for the comparison. These were specially selected because of their high humus content, in order to subject the methods to an extremely severe test. The results are given in table 1, from which the following conclusions may be drawn:

The potassium permanganate method is the most efficacious in the destruction of organic matter, next comes oxidation with sodium hypobromite; H_2O_2 treatment is the least effective.

All three methods of determining organic matter bring out the characteristic differences in the various treatments.

$KMnO_4$ completely decolorizes the soil, though it might still contain a small amount of organic carbon.

TABLE 1

Soil organic matter unoxidized after various treatments as checked by three methods of estimation

	PERCENTAGE OF CARBON			
SOIL NO.	Original soil	H_2O_2 treated	Hypobromite treated	$KMnO_4$ treated
	per cent	per cent	per cent	per cent
Colorimetric estimation				
13	2.26	1.98	0.0	0.0
76	9.5	2.98	2.03	0.0
77	6.3	3.0	1 65	0.0
78	3.5	1.8	1 70	0 0
79	2 98	2 80	2 58	0 0
81	8 38	2.78	1.9	0.0
82	5.5	2 5	2.3	0.0
86	8.38	3 26	1.9	0 0
87	9.1	2.34	1.6	0.0
Walkley and Black (4) method				
13	2.07	0.35	0.25	0.0217
76	8.4	4.89	2 6	1.27
77	5.5	1.5	0.73	0.99
78	5.1	1 12	0.75	0.1044
79	4.7	1.14	0.92	0.87
81	7.5	4.14	2.6	1.31
82	7.3	4.5	1.49	1.70
86	7.8	4.16	1.55	0.685
87	9.9	4.9	1.30	0 860
Loss on ignition				
13	11.7	11.9	9.6	8.4
76	38.0	19.89	16.7	6.3
77	14.5	7.1	6.1	4 3
78	12.42	5.26	5.6	4.1
79	11.8	5.2	6.5	4.7
81	37.8	18.69	17.3	6.1
82	18.6	7.2	9.7	4.8
86	33.6	12.75	7.9	4.5
87	32.0	14.2	7.9	4.5

SUMMARY

Three methods of destroying organic matter in the preliminary treatment of soils for mechanical analysis have been compared.

The alkaline potassium permangate method was most effective, the hypobromite method second, and the hydrogen peroxide method least effective.

REFERENCES

(1) CHAKRABORTY, J. N.   1935   The mechanical analysis of lateritic soils: III.   A new method using alkaline permanganate for oxidation of organic matter.   *Indian Jour. Agr. Sci.* 5: 41–50.

(2) ROBINSON, G. W.   1933   The dispersion of soils in mechanical analysis.   Imp. Bur. Soil Sci. Tech. Commun. 26.

(3) TROELL, E.   1932   The use of sodium hypobromite for the oxidation of organic matter in the mechanical analysis of soils.   *Jour. Agr. Sci.* 21: 476.

(4) WALKLEY, A., AND BLACK, I. A.   1934   An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method.   *Soil Sci.* 37: 29.

AMMONIUM AND NITRATE NITROGEN NUTRITION OF BARLEY AT DIFFERENT SEASONS IN RELATION TO HYDROGEN-ION CONCENTRATION, MANGANESE, COPPER, AND OXYGEN SUPPLY[1]

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INTRODUCTION

The relative merits of nitrate and ammonium salts as sources of nitrogen for higher plants have been the object of numerous investigations in recent years. The extensive literature on the subject has been dealt with recently in a number of comprehensive reviews (3, 9, 10, 17, 18, 19, 20, 22).

There is ample evidence at present to indicate that ammonium nitrogen can be absorbed and utilized directly by many species of higher plants (4, 14, 16, 23; also cf. 3, 9, 10, 17, 18, 19, 20, 22). Much of this evidence has been made available through investigations in which careful attention was given to the effects of ammonium and nitrate salts on the hydrogen-ion concentration of the culture medium. When ammonium salts are supplied as the only source of nitrogen for growing plants, the reaction of the culture medium becomes strongly acid in a relatively short time; nitrate salts, on the contrary, tend to shift the reaction of the culture medium in the direction of neutrality or alkalinity. The tendency in modern investigations on the relative suitability of these two sources of nitrogen has been to control experimentally the reaction of the culture medium, guarding against shifts in the pH traceable to the "physiological acidity" of ammonium or the "physiological alkalinity" of nitrate salts (9, 17, 20, 22).

These studies, although demonstrating the suitability of ammonium nitrogen for many plants, have not led to a general agreement regarding the relation of hydrogen-ion concentration to the utilization of these two forms of nitrogen (17). The conclusions from many of these investigations are not directly comparable in view of differences in the species of plants used and in experimental technics with their variable degrees of control over hydrogen-ion concentration and other environmental variables.

Various investigators (11, 12, 21) have recently questioned the value of interpreting plant responses in terms of the external hydrogen-ion concentration treated as an isolated variable. Evidence is available from diverse

lines of experimentation that the effects of hydrogen-ion concentration cannot be profitably appraised without regard to other factors of the cultural and climatic environment. The effect of the reaction of the culture medium on growth is modified by other variables, such as oxygen supply and temperature around the roots, and seasonal climatic fluctuations which influence the rate of growth and metabolic activities of plants. Furthermore, the composition of the culture medium may be altered by changes in reaction; at an alkaline reaction a number of elements will be precipitated and rendered less available for immediate use of the plant.

SCOPE OF INVESTIGATION

The object of this investigation was to determine the effects of season, hydrogen-ion concentration, aeration of the culture medium, and certain metals in minute quantity on the growth and nutrition of barley plants, comparing ammonium and nitrate salts as sources of nitrogen. The culture solution technic was used in these studies, since it made possible the most satisfactory degree of control of the variables mentioned. The interaction of these factors was considered by studying the effects of reaction at various seasons and the inter-relation of season, aeration, and concentration of "microelements," such as manganese.

This investigation was concerned not only with the influence of the factors mentioned on the growth and development of plants as reflected in yields but also with the effect of the various treatments on the chemical composition and several characteristics of plant sap, such as conductivity, pH, and total sugars. This paper is limited to the consideration of the effects of the various factors on plant growth. The discussion of the results of the quantitative analyses of plant tissues is reserved for another paper.

To increase the reliability of the interpretation of the observed responses, a relatively large number of plants (generally 64) was used for each treatment. In developing a suitable experimental technic for studying the effects of hydrogen-ion concentration, care was taken to avoid differences in the composition of the culture solution which may result from changing the reaction. The culture solutions of the lowest and highest pH investigated had the same concentrations of the important component ions, except for H, OH, and Na and SO_4 ions derived from the H_2SO_4 and NaOH used in adjusting the reaction.

METHODS OF EXPERIMENTATION

The relation of hydrogen-ion concentration to the composition of the culture solution

The effect of hydrogen-ion concentration on the culture medium, as distinguished from its direct effect on the plant, becomes an important consideration whenever the growth of plants is studied over a wide range of pH. The usual procedure used in such studies consists in selecting a culture solution of

a definite composition and then making it acid or alkaline by the addition of acids or bases. The composition of the culture solution, however, may be altered in respect to components other than just hydrogen ion by changing the initially acid reaction to an alkaline one. At alkaline reactions, considerable amounts of calcium, phosphorus, and other elements precipitate to an extent consistent with the solubility products of the precipitated salts. It is true that as soon as the very small amounts of these elements present in solution are absorbed by the plant, additional amounts dissolve, but only to the extent determined by the solubility products. Therefore, at alkaline reactions, the plant would grow in more dilute solutions of several essential elements than at acid reactions. High acidity may result in dissolving from certain types of culture vessels small but important amounts of such elements as copper, manganese and zinc. In soils similar effects of acidity and alkalinity may be much more complex and may have a profound effect on the welfare of the plant.

In this investigation, the composition of the culture solution was maintained

TABLE 1

Composition of culture solution used

	NH$_4$ SOLUTIONS	NO$_3$ SOLUTIONS
MgSO$_4$....................................	0.003M	0.003M
K$_2$SO$_4$......	0.0015M	0.001M
Ca(H$_2$PO$_4$)$_2$	0.002M	0.0015M
KH$_2$PO$_4$....	......	0 001M
CaSO$_4$	0 0015M	......
(NH$_4$)$_2$SO$_4$	0 002M	... .
Ca(NO$_3$)$_2$....	.	0 002M

relatively constant throughout the pH range studied. This was achieved by determining what concentration of the various ions it is possible to maintain at *the highest pH* investigated and adopting these concentrations for the entire range of pH.

The pH range from 4 to 6.7 was selected for study. The selection of pH 6.7 for the lowest hydrogen-ion concentration permitted the inclusion of a rather liberal supply of calcium and phosphate in the culture solution. The plants were grown in solutions maintained within narrow limits at pH 4, pH 5, pH 6, and pH 6.7, respectively. The composition of the culture solution used is given in table 1.

Iron was added as ferric tartrate, 0.62 cc. per liter of 0.5 per cent solution, thrice weekly. No microelements, such as zinc, copper, boron and manganese, were supplied in addition to the impurities contained in the salts used, unless otherwise specified. The experience of this laboratory over a period of years has shown that these impurities are adequate, under ordinary conditions, for the growth of barley plants.

The solutions were adjusted to the desired pH by adding N H_2SO_4 or N NaOH. Distilled water was used throughout in making up solutions.

Maintenance of a constant reaction

The initial reaction of the culture solution was maintained within relatively narrow limits by having a large volume of culture solution in relation to the number of plants. Changes in reaction and composition of the culture solution produced by the metabolic activities of growing plants were distributed over a large volume of solution and thus rendered quantitatively unimportant. The general effectiveness of this method was further enhanced by frequent renewals of the culture solution (once a week) and by provision of a suitable stirring by rapidly bubbling air through the solution to bring about a circulation of the culture solution within the culture vessel.

The initial pH was generally maintained within ± 0.1 pH. Large plants growing in ammonium solutions at times lowered the pH in one day beyond the limits indicated. The reaction was adjusted daily, when necessary. All pH measurements were made by the use of quinhydrone or glass electrodes.

Plants used

The Sacramento variety of barley, *Hordeum vulgare*, immune to mildew attack under local greenhouse conditions, was used in all experiments. The seeds were germinated in tap water according to the procedure described by Hoagland and Broyer (12). Seven-day-old seedlings were transplanted into tanks containing the nutrient solutions and grown in the greenhouse for 5 weeks. The containers were large iron tanks, 30 by 30 by 8 inches, 115 l. capacity, coated with asphalt paint, which was proved to be not toxic to plants. The cover of each tank held 64 corks, each cork supporting one plant (plate 1). In certain cases, smaller tanks containing 40 l. of culture solution and supporting 12 plants were used instead of the large ones.

The fresh and dry weights were expressed generally as averages of 64 plants. For fresh weight determinations, the roots were excised and the shoots weighed directly. The roots were centrifuged before being weighed, as described previously (12). Thirty-two shoots and roots from each treatment were dried at 56°C. for 72 hours or longer, and the percentage of moisture found was used in computing the average dry weight of the 64 plants.

Aeration and stirring

The effect of aeration on salt accumulation by roots has been recently discussed by Hoagland and Broyer (12). In the present investigation aeration served not only as a means of maintaining a high oxygen tension around the roots but also as an effective stirring device, mixing the solution within the tank and thus materially assisting in maintaining a uniformity of reaction and composition.

In studying the effects of hydrogen-ion concentration, it is well to consider

the reaction of the culture medium in the zone directly adjacent to the absorbing root surfaces. The absorbing root cell is in a dynamic equilibrium with the immediately adjacent zone of the culture medium and continuously alters its composition and reaction. The reaction in this zone may be different from that a short distance away from the roots. The presence of a suitable stirring device aids materially in the rapid elimination of such gradients.

The aeration technic used was that developed by Furnstal and Johnson (6). Forced aeration was provided in all experiments unless otherwise indicated. In cases where aeration was omitted, the treatment was designated as "no air." The "no air" treatment means that forced aeration was withheld but in no way implies an approach to an anaerobic condition. The use of shallow tanks with loosely fitting covers permitted access of air to a large surface, providing an oxygen supply even in the tanks without forced aeration superior to that usually available in jars or crocks.

Effect of season

The effect of hydrogen-ion concentration in the range from pH 4 to pH 6.7 was studied at two seasons: in the spring from February 28 to April 2, and in the fall from October 10 to November 14. Hereafter the plants will be referred to as spring and fall grown, respectively.

The effects of microelements and aeration were studied with plants grown at pH 6. The influence of manganese was tested in the spring and fall seasons; that of copper during the spring only. The response of barley plants grown at a constant pH of 6 to ammonium and nitrate nitrogen and the relative importance of forced aeration for these two sources of nitrogen were studied at several different times of the year.

Nitrification in ammonium culture solutions

The possibility of nitrification of ammonium nitrogen is a complicating factor in studying ammonium assimilation by the plant. This consideration alone would render the use of a soil undesirable for this kind of study. The ammonium solutions in which plants were grown were tested periodically for the presence of nitrates by means of the diphenyl-benzidine reagent. The tests were always negative. The possibility existed that small amounts of nitrate may be formed and immediately absorbed by the plants, thus escaping detection. In order to check this point, identical culture solutions were allowed to stand for a week in tanks not containing plants, and the solutions were then tested for nitrates. These tests were also negative.

Other observations also justify the conclusion that under the experimental conditions used no measurable nitrification took place. Distinctly different ammonium and nitrate plants[2] were obtained as far as general growth habit,

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[2] Hereafter, plants grown in culture solutions having ammonium as a source of nitrogen will be referred to as ammonium plants, and plants grown in nitrate-containing solutions as nitrate plants.

inorganic composition, and plant sap characteristics are concerned (1).   No
nitrate nitrogen could be found in ammonium plants.

PRESENTATION OF EXPERIMENTAL RESULTS

The mean fresh and dry weights of roots and shoots were taken as criteria
of plant growth.   Lack of space does not permit the complete citation of both
the fresh and dry weight data, which showed, however, a relationship.

The use of a large number of plants in each treatment decreased the error
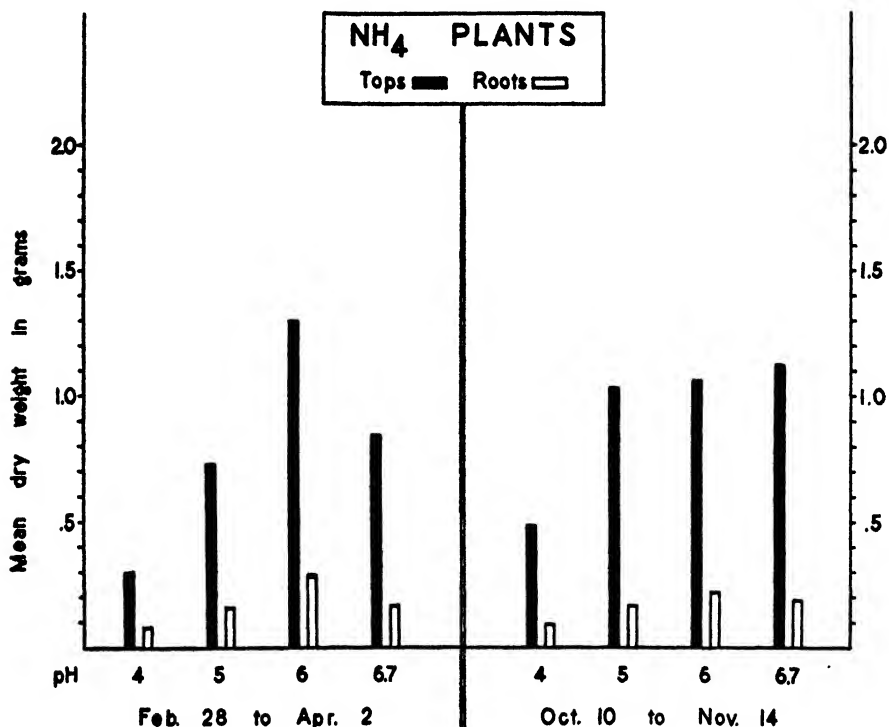
FIG. 1. THE EFFECT OF HYDROGEN-ION CONCENTRATION ON AMMONIUM PLANTS AT TWO
SEASONS OF THE YEAR.   AVERAGES OF 64 PLANTS

caused by variability in the seed.   The seedlings were selected for uniformity
at time of transplanting to the respective tanks.

In harvesting, all the plants representing one treatment were weighed
collectively.   The variability within a tank was tested in a preliminary experi-
ment by recording the weights of individual plants at pH 4 and pH 7 when
supplied with nitrate nitrogen.   Thirty plants representing each treatment
were weighed individually.   The arithmetical means for shoots from the
nitrate pH 4 treatment was 3.01 gm. and the standard deviation 0.52; the
mean and standard deviations for the shoots of the nitrate pH 7 treatment were

5.95 and 1.12, respectively. It will be noted that the tables, figures, and photographs show for the most part large differences between the respective treatments, reaching in several instances as much as several hundred per cent. The general plan of the investigation afforded an opportunity, in a number of cases, to test the validity of the observed responses by repeating a given experiment several times. The principal conclusions presented are based on magnitudes far exceeding those assignable to errors of variability in the plants used.
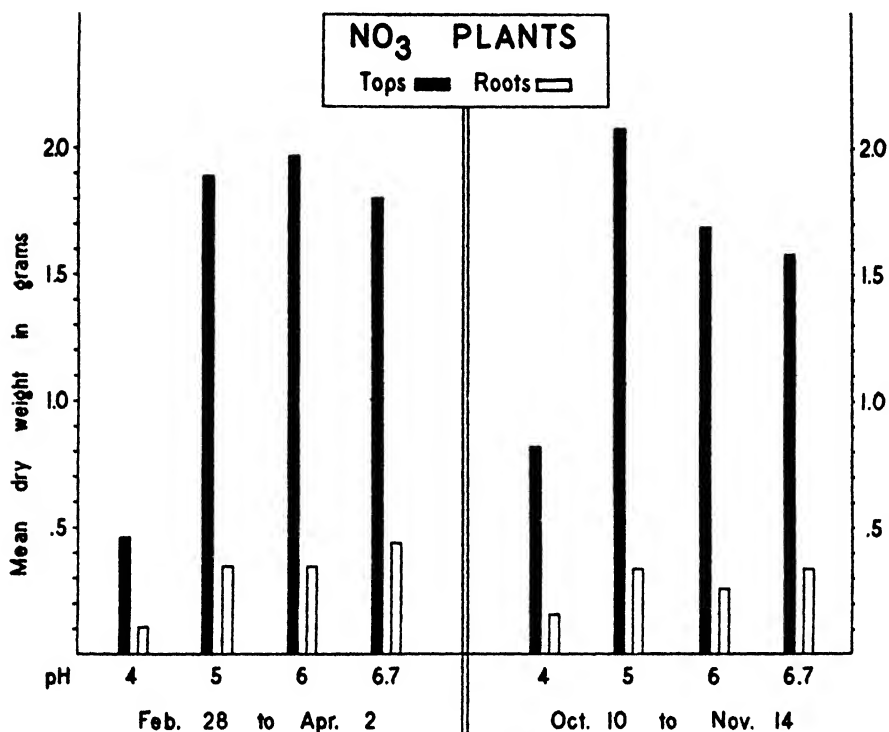
FIG. 2. THE EFFECT OF HYDROGEN-ION CONCENTRATION ON NITRATE PLANTS AT TWO SEASONS OF THE YEAR

INFLUENCE OF HYDROGEN-ION CONCENTRATION ON THE AMMONIUM AND NITRATE NITROGEN NUTRITION OF BARLEY

Effects of different hydrogen-ion concentrations at two seasons

Plants were grown in the spring and fall in culture solutions supplied with either ammonium or nitrate nitrogen, which were maintained at pH 4, 5, 6, and 6.7, respectively. The composition of the culture solution and the length of the growth period were similar during the two seasons. Forced aeration was provided in all cases. The mean dry weights of the plants are given in figures 1 and 2.

At pH 4 definite injury was found in both the ammonium and nitrate plants at either season, as indicated by the yield data. The plants grown at this hydrogen-ion concentration had a stunted and sickly appearance, pale shoots, and definitely injured root systems. The nitrate plants at pH 4 made more growth, however, than the ammonium plants.

Reaction above pH 4 had different effects at the two seasons on the ammonium and nitrate plants. In the spring the ammonium plants grew best at pH 6, excelling those grown at pH 5 and pH 6.7 (fig. 1). The nitrate plants grown in the spring failed to show a definite optimum at any hydrogen-ion concentration above pH 4. There was an indication of a slight depression in growth for nitrate plants at pH 6.7 (fig. 2).

These relationships between hydrogen-ion concentration, ammonium and nitrate, and the growth of plants appeared to be reversed in the fall. Ammonium plants grew equally well at pH 5, pH 6, and pH 6.7, whereas nitrate plants at pH 5 excelled those grown at pH 6 and pH 6.7 (fig. 1 and 2).

The results demonstrate the importance of season in studying the effect of hydrogen-ion concentration, within a wide range, on the utilization of ammonium and nitrate nitrogen by barley plants. When the reaction was maintained at pH 4, both the ammonium and nitrate plants were definitely injured in the spring as well as in the fall. This reaction then, may be regarded as sufficiently acid to be injurious to barley plants regardless of the season of the year and the form of nitrogen included in the culture solution. On the other hand, the growth of plants in the range from pH 5 to pH 6.7 with either ammonium or nitrate nitrogen was distinctly affected by season. In the spring the nitrate plants grew equally well, within limits of error, throughout this range of hydrogen-ion concentration, whereas the ammonium plants had an optimum growth at pH 6. In the fall the ammonium plants did not differ significantly in growth throughout this range of reaction, but the nitrate plants grew best at pH 5. Were the investigations limited to the spring season, it might have been concluded that the reaction of the culture solution, within relatively wide limits, has no marked effect on nitrate plants but produces a definite effect on ammonium plants. The reverse conclusion might have been reached from a study conducted in the fall.

It is impossible on the basis of these data alone to seek a more specific correlation between the responses noted and some particular factor of the climatic environment, such as temperature, length of day, intensity and quality of light, all of which vary with the season. It may be pointed out, without attempting to evaluate its importance, that in the spring the length of day progressively increased as the plants grew older, whereas in the fall the length of day progressively decreased. It is probable also that the photosynthetic activity of the plant was different at the two seasons. Hoagland (11) attributed seasonal differences in the nature of the root system and the quantitative relation of root to shoot, observed in experiments with barley

plants, chiefly to changes in the intensity and quality of light. The results reported in this paper indicate that these seasonal factors, irrespective of the mechanism by which they influence the plant, were of sufficient importance to modify the effects of hydrogen-ion concentration.

The pronounced injury consistently observed in all plants grown at pH 4, which was in sharp contrast to the healthy condition of the plants grown at pH 5 and above, suggests the existence of some critical hydrogen-ion concentration for barley grown in culture solution, lying between pH 4 and pH 5, below which toxicity occurs. This demonstrates the importance of using proper safeguards in maintaining the initial reaction constant in studies of the present type. The "physiological alkalinity" of nitrate salts may very readily change an initially toxic acid reaction into a physiologically tolerable one, whereas conversely, the physiological acidity of ammonium salts may quickly alter an initially favorable reaction into an injuriously acid one.

A comparison of figures 1 and 2 reveals that the nitrate plants made more growth during the spring and fall seasons than the ammonium plants, at every hydrogen-ion concentration investigated. Experiments were undertaken to compare the two sources of nitrogen at other seasons to see if this apparent superiority of nitrate nitrogen is maintained at all seasons of the year. Since it was not practicable to repeat the experiments over the entire range of pH, one hydrogen-ion concentration was selected at which the ammonium and nitrate plants were found to grow well.

Effect of season on plants grown at a constant hydrogen-ion concentration

In these and subsequent experiments, the plants were grown at a hydrogen-ion concentration maintained as nearly constant as possible at pH 6. The relative growth made by the ammonium and nitrate plants is graphically presented in figure 3. The nitrate plants were definitely superior to the ammonium plants in the early spring, but as the season progressed the differences between them diminished. In June the ammonium plants were slightly, though perhaps not significantly, better than the nitrate plants. The nitrate plants grew better in the October-November period, growing conditions at that period being still favorable at Berkeley, California. The unfavorable growing conditions in the November-December period resulted in a marked depression of growth of both the ammonium and nitrate plants. In the spring of the following year, the nitrate plants showed the same relative advantage over the ammonium plants as the year before.

Figures 1, 2, and 3 show the influence of seasonal variations on the response of plants to hydrogen-ion concentration. It will be shown presently that the responses of plants to other factors in the culture medium environment are also affected by season.

The dried plant tissues were analyzed for Ca, Mg, K, N, and PO_4. The ammonium plants were found to be consistently higher in phosphate and lower

in bases than the nitrate plants (1).   These chemical differences persisted throughout the pH range investigated.

EFFECT OF AERATION ON AMMONIUM AND NITRATE PLANTS GROWN AT PH 6 AT DIFFERENT SEASONS OF THE YEAR

It became apparent that the varied responses of barley plants to ammonium and nitrate nitrogen cannot be explained solely in terms of hydrogen-ion concentration.   The investigation led to the discovery of other factors which profoundly affect the utilization of these two forms of nitrogen by barley.

In the study of the effects of hydrogen-ion concentration, forced aeration
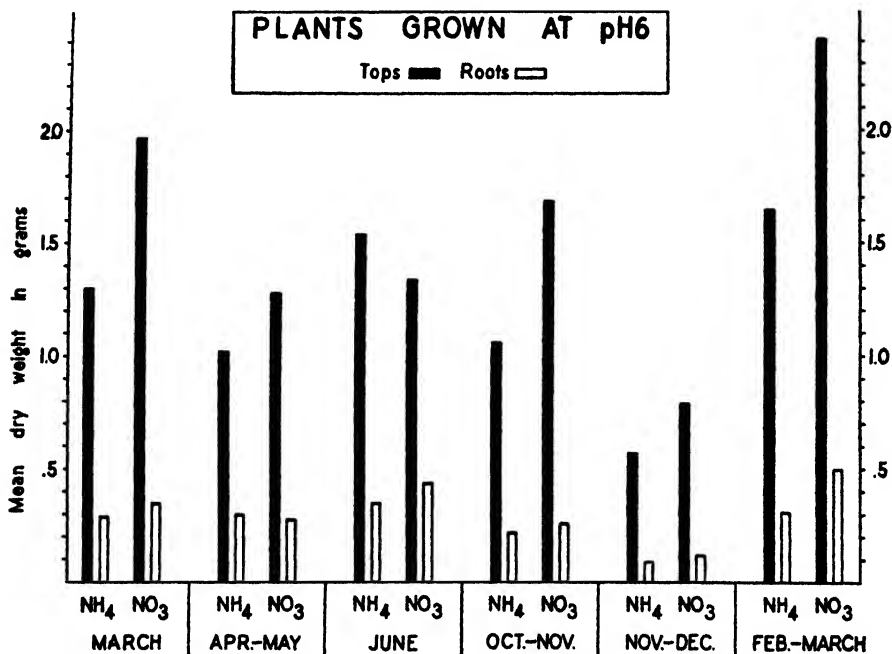
FIG. 3. EFFECT OF SEASON ON THE GROWTH OF AERATED NITRATE AND AMMONIUM PLANTS AVERAGES OF 60 PLANTS

was generally supplied to all ammonium and nitrate plants.   It was noted, however, that the omission of forced aeration affected the ammonium and nitrate plants differently, and this differential effect of aeration on nitrate and ammonium plants grown at pH 6 was studied at several seasons.   The results are presented in figures 4 and 5.

Figures 4 and 5 show that the lack of forced aeration, although strikingly limiting the growth of ammonium plants, hardly affected the total growth of nitrate plants.   This differential effect of aeration was tested and found to persist throughout the year.   The influence of season on the effect of aeration is illustrated in figure 4.   The depression in the growth of ammonium plants

from lack of oxygen was most pronounced when climatic conditions were favorable for growth. In the November-December period, aeration had little effect on the ammonium plants. At that season, growth was limited by the climatic environment; hence the favorable change in the culture medium resulting from aeration, demonstrated at other seasons, was not reflected in improved growth.

The non-aerated ammonium plants invariably differed in appearance from the aerated ones. The shoots of the non-aerated plants were pale and stunted,
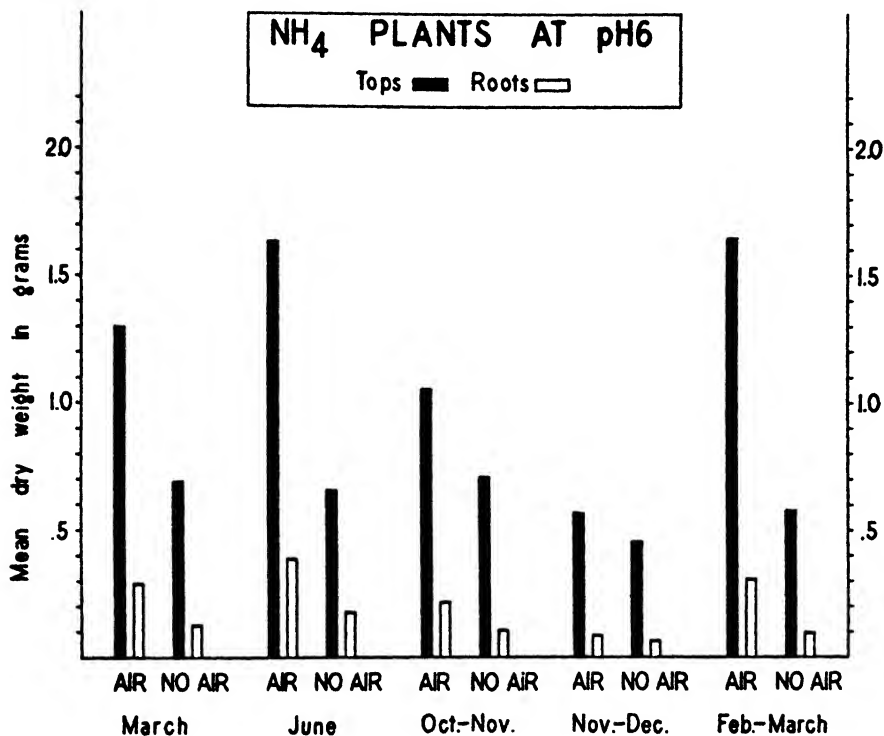
FIG. 4. EFFECT OF AERATION ON THE GROWTH OF AMMONIUM PLANTS AT DIFFERENT SEASONS OF THE YEAR. AVERAGES OF 60 PLANTS

whereas the aerated ones grew vigorously and had a normal green color. The roots of the non-aerated plants made little growth, in contrast to the long roots of the aerated plants. Aeration had comparatively little influence on the total amount of growth (dry weight yields) made by the nitrate plants (fig. 5). The aerated nitrate roots, however, differed from the non-aerated in the general habit of growth as described by Bryant (2). The shoots had a dark green color and were of similar healthy appearance in both treatments.

It will be noted that forced aeration not only supplied the solution with oxygen but acted as a stirring device as well. Consequently the depression

in growth of non-aerated ammonium plants could have been attributed to the absence of stirring and the resulting formation of localized zones of low hydrogen-ion concentration around the absorbing root surfaces, which were not eliminated rapidly enough. This explanation was rendered improbable by the experimental finding that the addition of small amounts of certain metals improved strikingly the growth of ammonium plants in non-aerated and unstirred culture solutions.
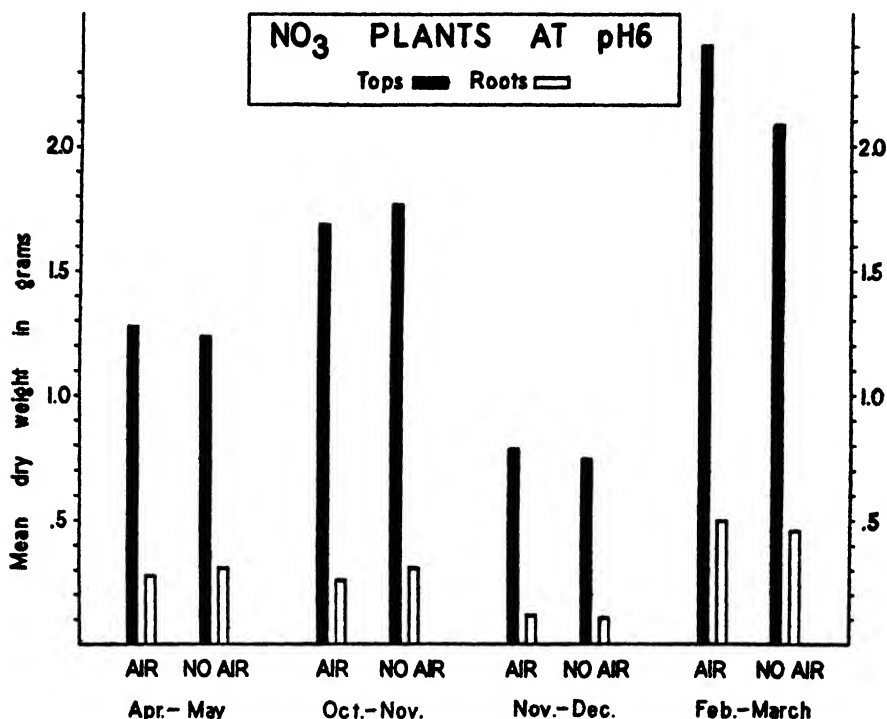
FIG. 5. EFFECT OF AERATION ON THE GROWTH OF NITRATE PLANTS AT DIFFERENT SEASONS OF THE YEAR. AVERAGES OF 60 PLANTS

EFFECT OF CERTAIN METALS IN MINUTE QUANTITY ON AMMONIUM AND NITRATE PLANTS AS MODIFIED BY SEASON AND AERATION

It has been previously mentioned that barley plants have been grown successfully in culture solutions in this laboratory without supplying such elements as manganese and copper in addition to the impurities contained in the usual nutrient salts or derived from culture vessels. The successful growth to maturity of barley plants justified our assumption that the chemically pure (C. P. grade) salts used in making the culture solutions contained adequate quantities of these microelements to satisfy the needs of the plants. In all such experiments, however, nitrogen was invariably supplied in the form of nitrates. In the present investigation, the effect of the addition of small quantities of certain metals on the growth of plants in solutions con-

taining nitrate nitrogen and those containing ammonium nitrogen was compared.

Manganese was added to the culture solution as $MnSO_4 \cdot 4\ H_2O$ to give a concentration of 0.5 p.p.m. Copper was added as $CuSO_4 \cdot 5\ H_2O$, molybdenum as MoO_3, and nickel as $NiSO_4 \cdot 6\ H_2O$, each to give a concentration of 0.05 p.p.m. in the respective culture solutions. For some of these experiments, as mentioned previously, smaller tanks were used instead of the larger ones.
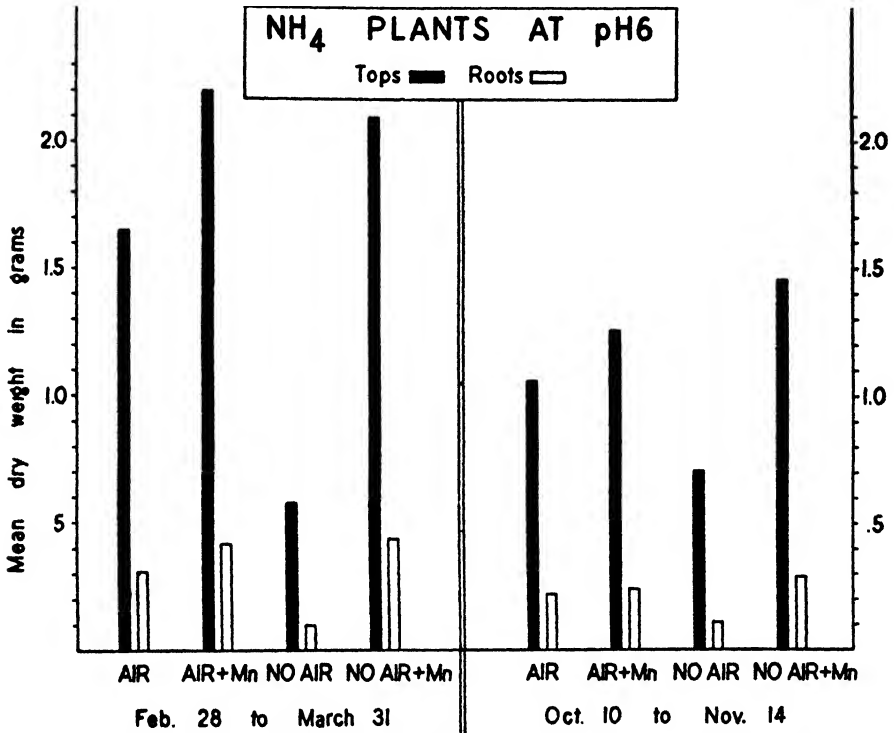
FIG. 6. THE EFFECT OF 0.5 P.P.M. OF MANGANESE WITH AND WITHOUT FORCED AERATION ON THE GROWTH OF AMMONIUM PLANTS AT TWO SEASONS

Effect of manganese

The effect of the addition of 0.5 mgm. of Mn to a liter of culture solution, with and without forced aeration, was observed during the spring and fall seasons. The response of the non-aerated ammonium plants to manganese was very striking. The increase in growth of shoots, as expressed as dry weight, was around 100 per cent in the fall and around 270 per cent in the spring. The addition of manganese to the aerated ammonium plants gave a noticeably larger improvement in the spring than in the fall. These results are presented in figure 6. The great improvement in the growth and appearance of the non-aerated ammonium plants resulting from the application of

manganese is illustrated in plates 2 and 3. The shoots of the manganese-treated plants had a healthy dark green color and large, well developed root systems.

The response of the non-aerated nitrate plants to manganese applications was not so marked as that of the ammonium plants. The former were excellent plants, far superior to the latter. The addition of manganese noticeably increased the growth of non-aerated nitrate plants in the fall, but not in the spring (fig. 7). The aerated nitrate plants were not improved at either season by the application of manganese. An interesting seasonal influence
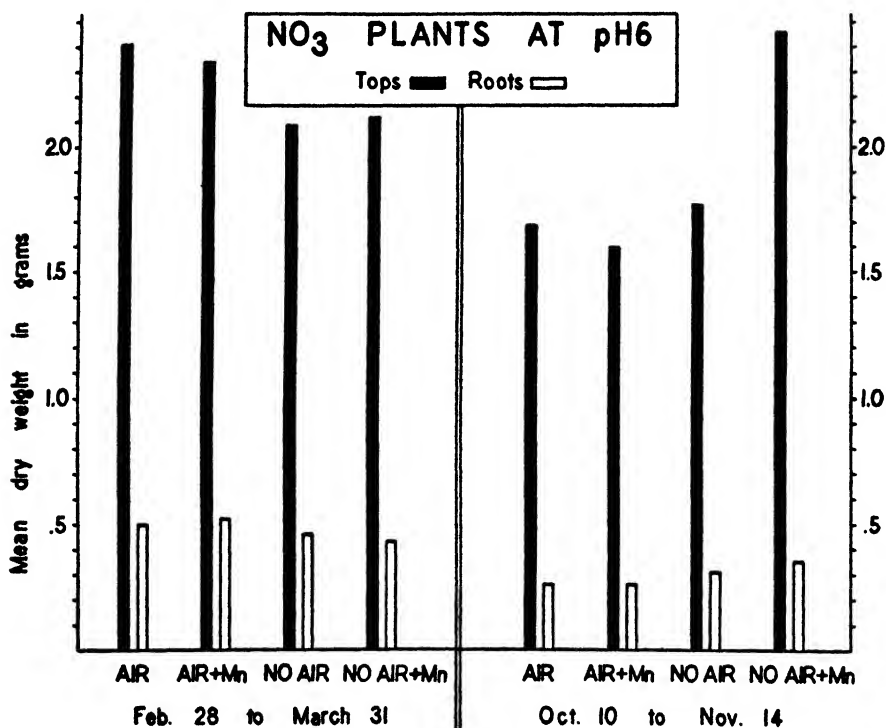

FIG. 7. EFFECT OF 0.5 P.P.M. OF MANGANESE WITH AND WITHOUT FORCED AERATION ON THE GROWTH OF NITRATE PLANTS AT TWO SEASONS

was noted in the effect that the combined treatment of aeration and manganese had on nitrate plants. In the fall, plants which received both aeration and manganese were inferior to those which received manganese without aeration. This was not the case in the spring (fig. 7). Aeration and additions of manganese were beneficial at that season but depressed growth in the fall.

The non-aerated nitrate plants, when treated with manganese, made more growth than the untreated ones, without evident alteration in the growth habits of shoot and root (plate 4). The addition of manganese to nitrate

culture solutions was correlated with the appearance of characteristic dark brown specks on the shoots. The specking was more pronounced when manganese was added to the aerated nitrate solutions. The ammonium plants similarly treated did not show these symptoms. It was demonstrated in other experiments not included in this report that the manganese concentration of the culture solution can be tripled before the specking occurs in the ammonium plants. A chemical analysis of the plants showed a consistently higher concentration of manganese in the shoots of nitrate, as compared with ammonium, plants.

Experiments with purified salts

The observed effects of manganese made it desirable to guard against the possibility that the nitrate culture solutions normally contained greater impurities of metals than the ammonium culture solutions. Such a condition could account for the lack of a comparable response to manganese applications in the nitrate series. The precaution was taken to purify the salts not common to the ammonium and nitrate culture media. A partial purification of this kind left in the two series of culture solutions only the impurities of metals contained in the salts common to both solutions. The $(NH_4)_2SO_4$ and $Ca(NO_3)_2$ were accordingly purified by removal of heavy metal impurities. In addition KH_2PO_4, which was used in the nitrate but not in the ammonium culture solution (table 1), was similarly purified. The method of purification will be described elsewhere.

The purification of the culture solutions, to the extent indicated, was employed for plants grown in the spring. The different responses of ammonium and nitrate plants to aeration and manganese persisted despite purification of salts. This would suggest that the responses described are primarily correlated with the particular source of nitrogen rather than with the differences in metal impurities carried by nitrate and ammonium salts.

A chemical analysis of the manganese-treated plants showed a higher phosphate content and a lower content of bases in the ammonium than in the nitrate plants (1). This effect of the source of nitrogen on the chemical composition of barley plants was found to remain unchanged by variations of season, hydrogen-ion concentration, or manganese.

Effect of copper

The effect of 0.05 p.p.m. of copper, with and without aeration, on the growth of ammonium and nitrate plants was studied in the spring. The results are presented in figure 8. The most striking response from copper was found in the case of the aerated ammonium plants. Here the increase in growth of shoots over the non-aerated plants, not treated with copper, was more than 400 per cent. Comparing the non-aerated ammonium plants, it was found that the copper treatment gave an increase in growth of about 140 per cent. The nitrate plants were likewise improved by the copper

application, but the improvement was not so striking, since the nitrate controls consisted of excellent plants as compared with the definitely injured ammonium control plants. The notable characteristic of copper-treated plants was their excellent root development, as is illustrated by the dry weights of roots (fig. 8). The addition of copper to the aerated ammonium plants gave an increase in dry weight of roots of about 180 per cent, and copper and aeration combined, an increase of about 870 per cent over the non-
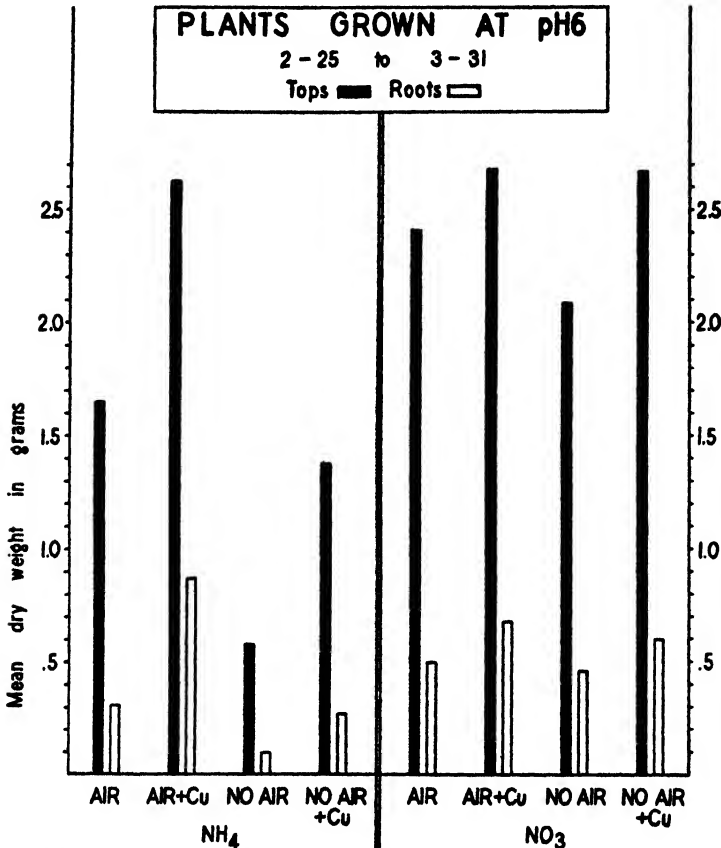
FIG. 8. EFFECT OF 0.05 P.P.M. OF COPPER ON PLANTS GROWN IN THE SPRING. AVERAGES OF 12 PLANTS

aerated ammonium plants with no copper added. A marked improvement in root growth was also observed as a result of adding copper to either the aerated or non-aerated nitrate solution. The effect of copper on non-aerated ammonium and nitrate plants is illustrated in plates 3 and 4, respectively.

The effect of copper was tested during the spring season only. A comparison of plant responses to manganese and copper during the same season is of interest. The outstanding point of similarity is the beneficial effect of these

two metals on the ammonium plants. It will be noted, however, that manganese was of greater benefit to ammonium plants in the non-aerated solutions, whereas copper was of greater benefit in the aerated culture solutions (fig. 6 and 8), producing the best ammonium plants ever grown in the course of this investigation. The application of copper and manganese to the non-aerated ammonium plants resulted in the production of different types of root systems (plate 3).

In the nitrate series, copper was of greater benefit in the non-aerated culture solution than in the aerated one. The copper-treated plants had a more extensive root system than plants grown without copper (plate 4). In general, copper was found to influence the growth of nitrate plants to a greater extent than manganese (fig. 7 and 8).

Effects of molybdenum, chromium, and nickel

The marked responses obtained from the application of copper and manganese suggested the desirability of testing the possible effects of other metals

TABLE 2

Effect of 0.05 p.p.m. of molybdenum, chromium, and nickel in non-aerated culture solutions on the growth of plants in early fall

(Mean dry weight of 12 plants)

TREATMENT	NH_4 SHOOTS	NO_3 SHOOTS	NH_4 ROOTS	NO_3 ROOTS
	gm.	*gm.*	*gm.*	*gm*
Control...	0.60	1.67	0.11	0.36
Molybdenum....	1.36	1.25	0 22	0.31
Chromium	1.41	1.50	0 23	0 24
Nickel....	1 36	1 19	0 25	0 29

in minute quantity. In a preliminary experiment, molybdenum, chromium, and nickel were added to the respective ammonium and nitrate culture solutions to give a concentration of 0.05 p.p.m. Plants were grown in early fall (September to October) in tanks containing 40 l. of solution and supporting 12 plants each. The results are presented in table 2. The purification of the culture solution as previously described was also carried out in testing the effect of these metals.

The controls in this experiment confirmed previously reported observations of the marked inferiority of ammonium plants in the absence of forced aeration. The added microelements increased by more than 100 per cent the dry weight of ammonium plants while depressing the growth of nitrate plants. The fresh weights not included here showed a parallel trend. A preliminary experiment with vanadium, titanium, and cobalt did not show marked effects on ammonium plants. It is not known, however, whether the arbitrarily selected concentrations were physiologically suitable or whether the elements were absorbed by the plant.

Could the effect of aeration on ammonium plants be attributed to impurities of metals contained in the aerators?

The aerators employed in studying the effect of aeration contained pieces of rubber tubing used to connect the glass elements (6, p. 193, fig. 4A). An experiment was carried out to test the possibility that the effect of aeration on ammonium plants could be attributed to impurities of metals yielded to the culture solution by the rubber tubing. An all-glass (Pyrex) aerator in which the units were sealed in series was used (6, p. 193, fig. 4B); in addition a rubber-glass aerator without air bubbling through it was placed in a non-aerated tank. The controls consisted of non-aerated tanks without aerators. The results are presented in table 3, which indicates that aeration, regardless of the type of aerator used, had a profound effect on the ammonium plants, causing an increase in dry weight of more than 150 per cent. It was shown previously that aeration did not influence the yield of nitrate plants.

Table 3 shows that a marked improvement in plant growth results from merely placing a rubber-glass aerator in a non-aerated ammonium tank. This

TABLE 3

Effect of type of aerator on the dry weight of plants grown in early fall (September–October)

(Mean dry weights of 12 plants)

TREATMENT	NH₄ SHOOTS	NO₃ SHOOTS	NH₄ ROOTS	NO₃ ROOTS
	$gm.$	$gm.$	$gm.$	$gm.$
Control..................	0.60	1.67	0.11	0.36
No air, rubber-glass aerator placed in tank .	1.06	....	0 20	..
Air, rubber-glass aerator......	1.66	.	0 33	...
Air, all glass aerator..	1 71	....	0.29	....

is interpreted as meaning that impurities in the rubber, important in quality and quantity, do enter the solution. Much larger yields were obtained, however, where forced aeration was applied *regardless* of type of aerator used. This suggests that the effect of aeration outweighs in importance that of the metal impurities derived from the rubber in the rubber-glass type of aerator.

DISCUSSION OF EXPERIMENTAL RESULTS

This investigation has sought to contribute to the understanding of the general problem of the utilization of ammonium and nitrate nitrogen by higher plants. This problem has been approached by studying a number of factors influencing the nitrogen nutrition of only one species. This limitation in the scope of the investigation, although admittedly precluding generalizations of validity for higher plants, has permitted the study of more variables than would be technically possible if several species of plants had been considered.

The results obtained indicate that season, hydrogen-ion concentration,

oxygen supply, and content of manganese, copper, and other metals in minute quantity profoundly affect the response of barley plants to ammonium and nitrate nitrogen. It was demonstrated that all these factors are interrelated in a complex manner, and their relative individual importance can be properly evaluated only by the employment of a suitable experimental technic.

The difference in the utilization of ammonium and nitrate nitrogen, as reflected in the growth of barley plants, cannot be satisfactorily explained in terms of a single variable. No support was found for the view which ascribes to hydrogen-ion concentration a position of unique importance in determining the response of plants to ammonium and nitrate nitrogen. The effects of hydrogen-ion concentration, within a wide physiologically tolerable range exclusive of extremes, were found to vary greatly at different seasons. At any given season and reaction other factors, such as aeration and minute amounts of metals, had a striking influence on the growth of ammonium plants, overshadowing in quantitative importance any changes produced by variations in hydrogen-ion concentration (cf. fig. 1 with fig. 4, 6, and 8). Complex reciprocal relationships, reflected in plant growth, were found between season, aeration of the culture solution, and the supply of metals. The results tend to support the view that a favorable culture medium needs to be defined with respect to a given set of climatic conditions. This seems to be particularly true of the concentration of microelements and of oxygen supply. It was shown in figure 7 that manganese plus aeration had a favorable effect on nitrate plants in the spring but depressed growth in the fall, when manganese without aeration gave best results.

The answer to the question whether plants grown in a culture solution with ammonium nitrogen can make as much growth as plants with nitrates as the sole source of nitrogen will depend on the experimental technic used. First, precautions must be taken not to permit the physiological acidity of ammonium salts to shift the reaction of the culture solution to an extremely low pH value, which, like pH 4 in these experiments, would be definitely toxic. If the solution is maintained within the relatively wide physiologically tolerable range of reaction, attention should be given to season, aeration, and the supply of minute quantities of certain metals. It was shown that ammonium plants grown in solutions without aeration are inferior to nitrate plants at all seasons studied (fig. 4 and 5). When aeration was provided, ammonium plants were found to make as much growth as nitrate plants at certain seasons only (fig. 3). They were still inferior to nitrate plants in the spring and fall. The addition of copper and manganese during these two seasons, however, produced ammonium plants equal in total amount of growth to nitrate plants (fig. 8, 6, and 7). Experiments not cited here indicate that the response of ammonium plants to manganese may increase when progressively larger applications, up to a certain point, are made as the plants grow older.

Several considerations suggest themselves regarding the use of the culture solution method for plant nutrition studies of the present type. The differ-

ent physical and chemical nature of the culture solution, as compared with a soil, may at times be responsible for obscuring certain nutritional relationships operating in a soil. The present study is a case in point. It was found that ammonium plants required a higher supply of oxygen and of microelements than the nitrate plants. Many soils contain adequate amounts of microelements and have favorable conditions of aeration. Thus, even in the absence of rapid nitrification of the soil, ammonium nitrogen may still give very good results. On the other hand, in a culture solution with ammonium salts as the sole source of nitrogen, the supply of microelements and aeration may be limiting. In such a case, the interpretation of the responses noted in terms of the nitrogen source alone would be misleading, since the omission of forced aeration and metals from the culture solution is not of equal consequence to ammonium and nitrate plants. Furthermore, metal impurities which may be contained in the nutrient salts become important. Ammonium salts from different sources might produce different effects, depending on the impurities of metals present. Where rigid purification of the salts employed is not technically feasible, the alternative course of adding to all culture solutions supplementary amounts of these microelements is suggested. The addition of an "A–Z" solution (13) would provide further protection by including traces of a large number of elements present in a soil but not yet shown to be essential for plant growth. The importance of aeration in culture solutions in relation to salt absorption by roots is discussed by Hoagland (11) and Hoagland and Broyer (12).

Nitrates are shown to be "safer" sources of nitrogen than ammonium salts in culture solution work, since they do not require such careful attention to reaction, aeration, and supply of metals. It does not follow, however, that a combination of ammonium and nitrate salts should not find wide application in ordinary culture solution work. The supplying of nitrogen from the two sources may have distinct advantages by providing, for example, a "physiological buffer" which would guard against rapid shifts in the reaction of the culture solution (24). It is further suggested that culture solution experiments including both ammonium and nitrate nitrogen may yield information which would assist more materially in interpreting responses of plants to ammonium fertilization in the field than experiments with ammonium salts as the only source of nitrogen. Under ordinary field conditions, ammonium is nitrified, and the soil solution contains both ammonium and nitrate nitrogen for some time after the addition of ammonium salts.

INFLUENCE OF NITROGEN CARRIERS ON METABOLIC ACTIVITIES OF PLANTS

Evidence is available from several diverse lines of investigation that nitrates may be of value to the plant in addition to serving as a source of nitrogen. Warburg and Negelein (26) found that the respiratory quotient of Chlorella increased, following absorption of nitrates, from the normal range of R.Q. =

1.00–1.04 to the range R.Q. = 1.3–2.0. Hamner (7) found a marked increase in the respiration rate from the application of nitrates to tomato and wheat plants, provided an initial carbohydrate reserve was available. Results from unpublished experiments in this laboratory have shown that the application of nitrates increased the rate of respiration of excised barley roots.

Meyerhof's computations (15), based on the results of Warburg and Negelein (26), show that nitrate is a very effective oxidizing agent: for every NO_3^- reduced to NH_4^+ two carbon atoms are oxidized with a release of 162,000 calories. Of the energy released by the oxidation of carbon to CO_2, only 30 per cent is used in the reduction of NO_3^- to NH_4^+.

Furnishing the plant with nitrogen exclusively in the reduced form of ammonium may be regarded in the light of the foregoing considerations as not merely a substitution of one source of nitrogen for another but as a deprivation of the plant of an oxidizing agent. It is suggested that the better results that are frequently obtained with nitrates in culture solutions may be due, in part at least, to these additional functions that nitrate may perform as it is reduced in the plant. If this view is accepted, then the beneficial effect on ammonium cultures of forced aeration and the addition of microelements may be attributed to the fact that these two modifications of the ammonium culture solution tend to compensate the plant for the oxidative function of nitrate. The increased oxygen supply and respiration rate of the roots which would result from the reduction of nitrate would be brought about in the case of ammonium cultures by the increased oxygen supply from forced aeration. It is further suggested that the effects of metals such as manganese and copper may be attributed to their catalytic function in the promotion of oxidation-reduction processes in the plant. It will be noticed that all the metals found beneficial for ammonium plants are capable of assuming several valence levels and would thus be chemically suitable for exercising a catalytic function in oxidation-reduction processes (25).

The possibility exists that nitrate nitrogen, by virtue of its being a rapidly absorbed anion or by its stimulative effect on root respiration, may bring about more effective absorption of metallic microelements than ammonium nitrogen. This might conceivably be important when the initial supply of microelements in the culture medium is low and may explain the less pronounced response of nitrate plants to applications of copper and manganese. This point is being considered in experiments now being conducted.

It is in no way implied that the sole function of copper and manganese in the plant is confined to their possible function in oxidation-reduction processes. Other functions are without doubt performed by these elements. By way of illustration, it may be mentioned that manganese applications were found to be correlated with higher concentrations of total sugars in plant sap. Recent experiments in this laboratory with other crops disclosed a similar effect of manganese. The generally similar responses obtained from the application

of a number of metals to ammonium plants raises the question to what extent, if at all, one element may "substitute" for another in the metabolism of the plant.

SUMMARY

Water culture methods are described for the study of ammonium and nitrate nitrogen nutrition of young barley plants grown at different seasons, with control of the variables of reaction, aeration, and concentration of certain microelements, particularly manganese and copper.

The results of the experiments show that hydrogen-ion concentration within a relatively wide range is only one of the variables which determine the effects of ammonium and nitrate nitrogen on plant growth, and it must be interpreted with reference to other variables mentioned above.

The effect of the external reaction, maintained at pH 5, pH 6, or pH 6.7, on the growth of plants was modified by season. In the spring the ammonium plants grew best at pH 6 but failed to excel at that reaction in the fall. The nitrate plants made best growth at pH 5 in the fall, but did not show any significant advantage at that reaction in the spring.

Plants grown in either ammonium- or nitrate-containing culture solutions, maintained at pH 4, showed definite injury during both the spring and fall seasons.

Growth of ammonium plants was affected extensively and favorably by either forced aeration of the culture solution or by the addition of manganese, copper, or certain other metals, without forced aeration. The proper adjustment of the culture solution with respect to these factors has made it possible to produce ammonium plants not inferior to nitrate plants at every season at which experiments were performed.

The increase in dry weight of ammonium plants obtained by the proper adjustment of aeration of the culture solution and the concentration of metals was as much as 400 per cent for shoots and 800 per cent for roots. The effects of these factors on the nitrate plants were relatively small.

Some general views of the possible functions of catalytic metals and aeration of roots in relation to the utilization of ammonium and nitrate nitrogen are discussed.

REFERENCES

(1) ARNON, D. I. 1937 Effect of ammonium and nitrate nitrogen on the mineral composition and sap characteristics of barley. (In preparation.)
(2) BRYANT, A. E. 1934 Comparison of anatomical and histological differences between roots of barley grown in aerated and in non-aerated culture solutions. *Plant Physiol.* 9: 389–391.
(3) CHIBNALL, A. C., AND PRYDE, J. 1930 *Chem. Soc. Ann. Rpt.* 27: 242–247.
(4) CLARK, H. E. 1936 Effect of ammonium and nitrate nitrogen on the composition of the tomato plant. *Plant Physiol.* 11: 5–24.
(5) ECKERSON, S. H. 1929 Properties of the nitrate reducing substance in plants. *Amer. Jour. Bot.* 16: 851–852.

(6) FURNSTAL, A. H., AND JOHNSON, B. 1936 Preparation of sintered pyrex glass aerators for use in water culture experiments with plants. *Plant Physiol.* 11: 189–195.

(7) HAMNER, KARL C. 1936 Effects of nitrogen supply on rates of photosynthesis and respiration in plants. *Bot. Gaz.* 97: 744–795.

(8) HOAGLAND, D. R. 1923 The absorption of ions by plants. *Soil Sci.* 16: 225–246.

(9) HOAGLAND, D. R. 1932 Mineral nutrition of plants. *Ann. Rev. Biochem.* 1: 618–636.

(10) HOAGLAND, D. R. 1933 Mineral nutrition of plants. *Ann. Rev. Biochem.* 2: 471–484.

(11) HOAGLAND, D. R. 1936 The plant as a metabolic in the soil-plant system. Essays in Geobotany in Honor of W. A. Setchell. University of California Press, Berkeley.

(12) HOAGLAND, D. R., AND BROYER, T. C. 1936 General nature of the process of salt accumulation by roots with description of experimental methods. *Plant Physiol* 11: 471–507.

(13) HOAGLAND, D. R., AND SNYDER, W. C. 1933 Nutrition of strawberry plants under controlled conditions. *Proc. Amer. Soc. Hort. Sci.* 30: 70–86.

(14) HUTCHINSON, H. G., AND MILLER, N. H. J. 1909 Direct assimilation of ammonium salts by plants. *Jour. Agr. Sci.* 3: 179–194.

(15) MEYERHOF, O. 1924 Chemical Dynamics of Life Phenomena, p. 93. J. B. Lippincott Co., Philadelphia, Pa.

(16) MOTHES, K. 1926 Ein Beitrag zur Kenntnis des N-Stoffwechsels höheren Pflanzen. *Planta* 1: 473–552.

(17) PARDO, J. H. 1935 Ammonium in the nutrition of higher green plants *Quart. Rev. Biol.* 10: 1–31.

(18) POLLARD, A. G., AND PRYDE, J. 1931 *Chem. Soc. Ann. Rpt.* 28: 240–243.

(19) POLLARD, A. G., AND PRYDE, J. 1932 *Chem. Soc. Ann. Rpt.* 29: 255–256.

(20) POLLARD, A. G., AND PRYDE, J. 1933 *Chem. Soc. Ann. Rpt.* 30: 320–323.

(21) STEWARD, F. C. 1935 Mineral nutrition of plants. *Ann. Rev. Biochem.* 4: 519–544.

(22) STEWARD, C. P., AND POLLARD, A. G. 1934 *Chem. Soc. Ann. Rpt.* 41: 349–352.

(23) TIEDJENS, V. A. 1934 Factors affecting assimilation of ammonium and nitrate nitrogen, particularly in tomato and apple. *Plant Physiol.* 9: 31–57.

(24) TRELEASE, S. F., AND TRELEASE, H. M. 1935 Changes in hydrogen-ion concentration of culture solutions containing nitrate and ammonium nitrogen. *Amer. Jour. Bot.* 22: 520–542.

(25) WARBURG, O. 1925 Iron, the oxygen carrier of respiration ferment. *Science* 61: 575–582.

(26) WARBURG, O., AND NEGELEIN, E. 1920 Über die Reducktion der Salpetersäure in grünen Zellen. *Biochem. Ztschr.* 110: 66–116.

PLATE 1

PLANTS GROWING IN TANKS
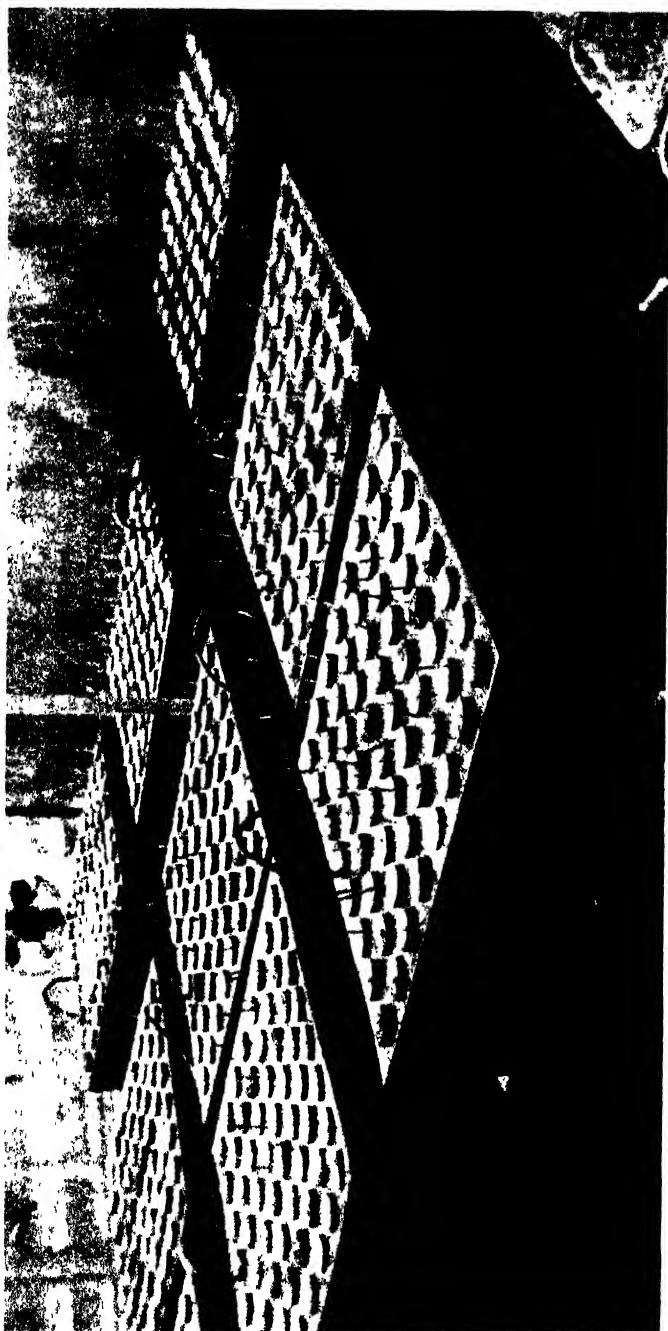
PLATE 2

EFFECT OF 0.05 P P M. OF MANGANESE ON NON-AERATED AMMONIUM PLANTS GROWN IN THE
FALL   (CONTROLS ON THE RIGHT)

D. I. ARNON
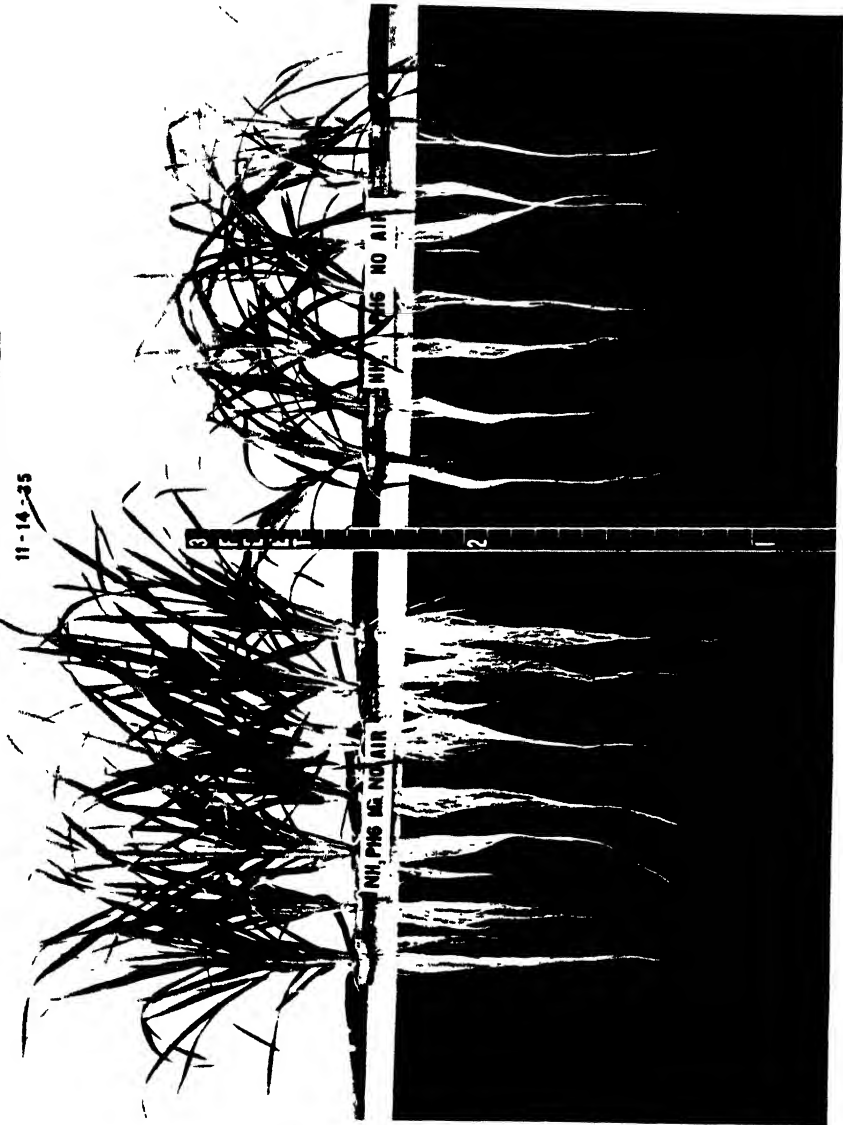
PLATE 3

EFFECT OF COPPER AND MANGANESE ON NON AERATED AMMONIUM PLANTS GROWN IN THE SPRING

PLANTS 42 DAYS OLD

3-31-36

NH, PH6 N₄ NO AIR

NH, PH6 NO AIR

NH, PH6 CU NO AIR
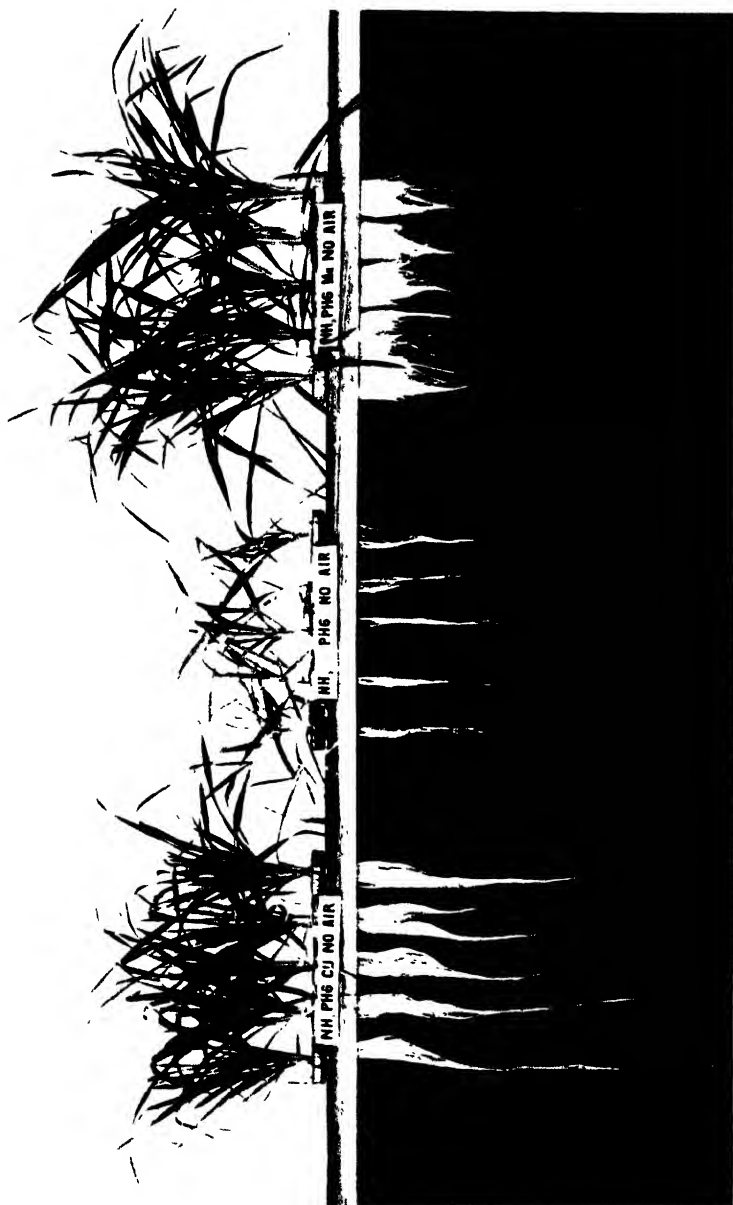
PLATE 4

EFFECT OF COPPER AND MANGANESE ON NON-AERATED NITRATE PLANTS GROWN
IN THE SPRING
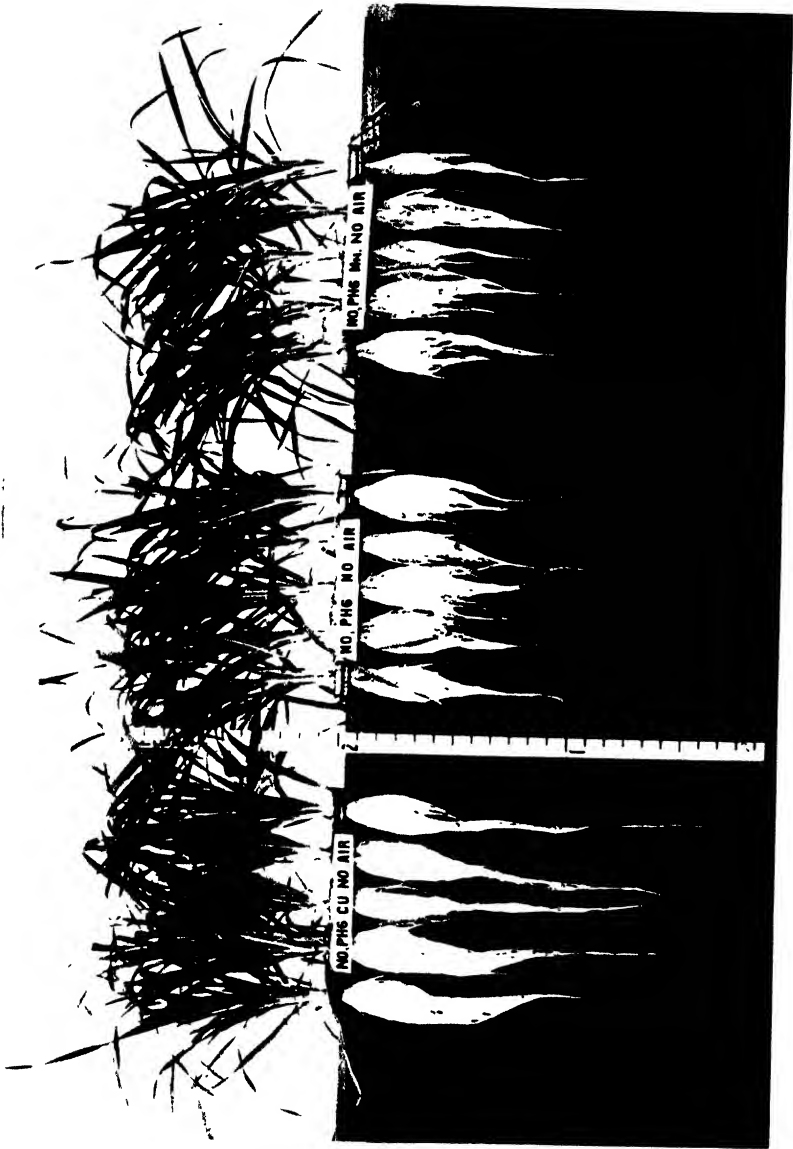
THE INFLUENCE OF HYDROGEN PEROXIDE TREATMENTS ON THE EXCHANGE CAPACITY OF MARYLAND SOILS[1]

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INTRODUCTION

Since the potential productivity of a soil is considered to be proportional to the total exchange capacity, any means of evaluating this capacity should be helpful in managing a soil. This is especially true when the two components of the exchange complex—organic and inorganic—are considered separately. Under normal conditions the inorganic complex is generally recognized as independent of man's influence. On the other hand, the organic portion may be varied considerably. This potential fertility level and the possibility of changing one part of it should make a knowledge of the relative amounts of the organic and inorganic exchange capacity of a soil a valuable aid in its treatment. From this viewpoint, a study to estimate the amounts of these two exchange complexes was made on a large number of Maryland soils. This paper reports the results of this investigation.

EXPERIMENTAL

Two hundred and forty samples, representing 31 series and 51 soil types in the state, were used in this study. These samples were obtained by the use of a soil auger, a soil probe, or a trowel. Each sample was a composite of a large number of samplings taken from the plowed layer of an area typical of the respective soil type. The field method of classifying soils was used to determine each soil type. At the time of sampling a record of the productivity, crop rotation, and fertilizer practices was obtained. These records indicate that these samples represent many degrees of fertility and several kinds of farming practices. In so far as it was practical, the number of samples taken for each soil type was in proportion to the total acreage of that soil type in the state. This ratio between the number of samples taken and the total acreage mapped applied particularly to each soil province.

When brought into the laboratory, these samples were air-dried, screened, and mixed. All gravel, stones, and organic fractions which did not readily pass through a 10-mesh screen were discarded. The exchange capacity of all these samples was measured before and after treatment with hydrogen

123

peroxide. The completeness of this oxidation, or organic matter removed, was estimated by total carbon determinations on the soils, following each exchange capacity measurement.

The method used for determining the total exchange capacity was similar to that proposed by Schollenberger and Dreibelbis (3). Fifty grams of soil was mixed with 100 ml. of N ammonium acetate (adjusted to pH 7.07). After this mixture had stood over night, it was transferred to a leaching tube and then leached with 500 ml. more of ammonium acetate. The soil was then washed free of ammonium acetate with 400 ml. of 80 per cent ethyl alcohol. After the alcohol leaching, the ammonia in the soil was displaced with 5 per cent sodium carbonate by aeration and absorption in 0.1 N sulfuric acid. The excess acid was titrated with standard sodium hydroxide, using the indicator proposed by Johnson and Green (2). The amount of ammonium displaced from the soil in the aeration was taken as a measure of the total exchange capacity and was calculated in terms of milliequivalents per 100 gm. of soil.

The organic matter was removed by treating 50 gm. of the soil with several applications of approximately 100 ml. of 6 per cent hydrogen peroxide. The treatment was made at room temperature in tall 400 ml. beakers to prevent loss by foaming. Because some of these soils contained a large amount of manganese, a few drops of glacial acetic acid were added to each sample when it was treated with fresh quantities of hydrogen peroxide. Each treatment of hydrogen peroxide was allowed to proceed until the reaction had ceased and the solution became clear. This reaction was considerably stimulated by periodic rotary shaking of the beakers. The clear liquid was decanted by suction, and new portions of hydrogen peroxide were added. These treatments were repeated until there appeared to be no more action between the soil and hydrogen peroxide. In most cases this required five to six treatments. In a few soils it was found that settling was very slow and never complete; to these beakers a drop or two of a saturated solution of aluminum sulfate was added to flocculate the colloids. The oxidized soil was then treated with ammonium acetate and the exchange capacity determined as for the unoxidized soil. After the ammonium had been determined on the soils by aeration, the resulting mixture of soil and sodium carbonate was neutralized with sulfuric acid, to prevent a loss of organic matter because of the alkaline treatment. The soils were then washed with water by decanting several times to remove the salts and the excess acid. After drying, they were passed through a 20-mesh screen and mixed. Total carbon determinations were made on each sample by a method similar to that proposed by Heck (1). The total carbon obtained by this method was transposed to organic matter by the use of the conversion factor 1.724. The results were calculated on the basis of the 50 gm. of soil originally used for the leaching and oxidizing.

The data obtained by this procedure are given for the five different provinces in tables 1–5. Within each province the soils are arranged according

TABLE 1

The influence of oxidation on the total base exchange capacity and organic matter content of Coastal Plain soils

SOIL NO.	SOIL TYPE	TOTAL EXCHANGE CAPACITY			ORGANIC MATTER		
		Unoxi-dized	Oxidized	Loss	Unoxi-dized	Oxidized	Loss
		m.e.	*m.e.*	*per cent*	*per cent*	*per cent*	*per cent*
	Collington						
236	Sandy loam	2 14	1 29	39 8	0 52	0 24	54 70
237	Fine sandy loam	5 83	2 35	59 7	0 85	0 19	77 60
238	Fine sandy loam	3 88	2.10	45 9	0 74	0 10	86 50
241	Fine sandy loam	5 63	4 65	17 4	0 95	0 18	81 00
242	Fine sandy loam	3.16	2 05	35 2	0 58	0.13	77.60
239	Very fine sandy loam	2 96	3 08	. . .	0 92	0 14	84 80
240	Very fine sandy loam	4 93	4 25	13.8	0 95	0 08	91 60
	Elkton						
1	Silt loam	2 07	0 99	52 17	0 88	0 09	90.19
2	Silt loam	2 33	1.16	50 21	0 93	0 07	92 40
3	Silt loam	3 41	1 96	42 52	1 34	0 15	88 80
4	Silt loam	2 56	2 22	13 28	1 40	0 22	84 40
202	Silt loam	4 57	2.72	40 50	1 06	0 14	86 80
203	Silt loam	4 68	2 74	41 50	1 08	0 30	72 20
211	Silt loam	5 10	1 96	61 60	1 24	0 32	74 20
216	Silt loam	3 31	2 05	38 10	1 68	0 40	76 20
220	Silt loam	3 80	3.79	0 30	2 07	0 59	71 50
221	Silt loam	2 42	2.06	14 90	1 06	0 25	76 40
5	Loam	3 38	1 21	64 20	0 83	0 07	91 60
6	Loam	2 36	1 12	47 50	0 86	0 09	89 60
	Keyport						
7	Silt loam	3 53	1.87	47 00	1 57	0.21	86 60
8	Silt loam	2 86	1.87	34 60	1 29	0 10	92 30
211	Silt loam	4 10	2 70	34 20	1 62	0 22	86 40
212	Silt loam	3 75	2 96	21 10	1 12	0 28	72 50
215	Silt loam	2 95	2 66	9 80	1 14	0 18	84 20
217	Silt loam	3 45	2 68	22 30	1 60	0 44	72 50
209	Loam	3 04	1 14	62 50	1 17	0 05	95 70
210	Loam	3 25	0 76	76 60	1 31	0 10	92 10
205	Fine sandy loam	2 64	0 91	65 50	0 88	0 24	72 90
208	Fine sandy loam	2 48	1 47	40 70	1 07	0 25	76 60
213	Fine sandy loam	2 10	0 54	74 30	1 10	0 15	86 40
214	Fine sandy loam	3 98	1 65	59 40	1 16	0 28	75 80
	Leonardtown						
222	Silt loam	2 58	1 87	27 50	1 22	0 28	77 00
223	Silt loam	2.67	1 70	36 30	1 07	0.18	83 40
243	Silt loam	3.12	2 12	32 00	1 00	0 31	69 00
244	Silt loam	3 49	1 77	49 30	1 16	0 15	87.10
245	Silt loam	3 94	1.75	55 60	1 30	0 19	85 50
246	Silt loam	3 55	2 01	43.3	0.82	0 25	69.20

TABLE 1—*Concluded*

SOIL NO.	SOIL TYPE	TOTAL EXCHANGE CAPACITY			ORGANIC MATTER		
		Unoxidized	Oxidized	Loss	Unoxidized	Oxidized	Loss
		m.e.	m.e.	per cent	per cent	per cent	per cent
	Portsmouth						
218	Loam	31.45	4.28	86.40	7.59	2.74	63.90
207	Loam	9.76	2.38	75.60	2.57	0 48	81.30
204	Loam	13.18	1.50	98 60	4.05	0.10	97 50
219	Loam	31.00	12.40	60 00	7.23	2.48	65.70
206	Fine sandy loam	9.47	1.29	86.40	1.71	0.33	80.70
209	Fine sandy loam	9.28	0.66	92.90	2.34	0.27	88.50
210	Fine sandy loam	15 53	1.49	90.20	5.28	1.18	77.60
	Sassafras						
11	Silt loam	3.15	2.10	33 30	1 10	0 28	74 60
12	Silt loam	2.26	2.10	7.10	1.15	0 19	83.50
13	Silt loam	2.32	1.88	19 00	1.15	0.14	87.80
14	Silt loam	2.92	2.01	31.20	1.21	0.14	89.40
15	Silt loam	2.67	2.16	19.10	1.34	0.12	91 00
16	Silt loam	3.51	2 76	21.40	2.10	0.17	91.90
17	Silt loam	3.23	0.55	73.70	1.17	0.21	82.00
18	Silt loam	3.78	1.86	50 80	1.38	0 24	82.60
19	Silt loam	3.46	1.16	66.50	0 96	0 17	82.30
20	Silt loam	2.96	1.38	53.40	0.95	0.17	83.20
21	Silt loam	2.65	2.54	4 20	2.12	0.43	79.80
22	Silt loam	2.77	2.30	15 80	1.69	0.21	87.60
23	Silt loam	3.07	2.27	23.10	1.95	0.41	79.00
24	Silt loam	2.67	1.98	25.80	1.21	0.10	91.70
25	Silt loam	2.51	1.90	24.30	1.60	0.16	90.00
26	Silt loam	2.80	2.75	1.80	1.62	0.22	86 40
27	Silt loam	2.57	1.74	32.30	1 64	0.21	87.20
28	Silt loam	2.79	1.21	56.60	1.86	0.19	89 80
29	Silt loam	2.52	0.93	63.1	1 48	0.19	88.20
30	Silt loam	2.86	1.57	45.10	1.47	0.10	93.20
31	Silt loam	3.13	2.13	31 90	1 43	0.16	88.80
32	Loam	1.65	0.64	61 20	0 84	0.07	91.70
33	Loam	·1.86	0.80	57.00	1 09	0 07	93.60
34	Loam	1.75	0.69	60.60	0.76	0.05	93 40
35	Loam	2.03	1.18	41.90	1.03	0.16	84.50
36	Loam	2.06	0 95	53.6	0 91	0.21	76.90
37	Loam	2.17	0.93	57.10	1.09	0.14	87.20
38	Loam	2.74	1.11	59.50	0.69	0.09	86.90
39	Loam	2.81	0 91	67.50	1.38	0.21	84.80
44	Sandy loam	1.51	0.08	94.70	0.69	0.09	87.00
45	Sandy loam	1.95	0.39	80.00	0.83	0.03	63.80
46	Sandy loam	1.97	0.86	56.30	0 67	0 19	71.60
47	Sandy loam	2.04	1.42	30.40	0.59	0.24	59.30
40	Loamy sand	1.97	0.83	57 90	0.67	0 09	86.60
41	Loamy sand	1.98	0.88	55 60	0.41	0.14	65.90
42	Loamy sand	1.77	0.88	50.30	0.34	0.16	53.00
43	Loamy sand	2.02	0.75	67.90	0.40	0.05	87.50

TABLE 2

The influence of oxidation on the total exchange capacity and organic matter content of Piedmont soils

SOIL NO.	SOIL TYPE	TOTAL EXCHANGE CAPACITY			ORGANIC MATTER		
		Unoxi-dized	Oxidized	Loss	Unoxi-dized	Oxidized	Loss
		m.e.	*m.e.*	*per cent*	*per cent*	*per cent*	*per cent*
	Ashe						
285	Gravelly loam	8.72	3.65	58 10	1.33	0 53	60.80
296	Gravelly loam	7.86	4.78	39 30	0.93	0.18	80 60
291	Stony loam	5.85	3.66	37.50	1.02	0 27	73 50
290	Loam	10.82	2.48	77.10	0.82	0 10	87.60
292	Loam	7.45	3.22	56.70	0 98	0 10	89.70
	Cardiff						
282	Slate loam	9.39	2.79	70 20	1 50	0 23	84 70
297	Slate loam	5 83	2 75	52.90	1.44	0 25	82.60
	Chester						
85	Loam	3.12	1 81	42 00	1.67	0 48	71.20
86	Loam	4.33	1.76	59 40	1 42	0.57	59.90
87	Loam	4.21	1.71	59 40	1 71	0 29	83 00
88	Loam	3.05	2.17	28 80	2 48	0.29	88 30
89	Loam	4.60	2 66	42 20	2.64	0 50	81.10
90	Loam	4 75	2 76	41.90	1.95	0 19	90.30
91	Loam	5 36	4.61	14 00	1.90	0.33	82.60
92	Loam	7 54	4.92	34.70	1 40	0 34	75.70
93	Loam	4.97	3.98	19 90	1 17	0.22	81.20
94	Loam	3 15	2.67	15 20	1 28	0 95	25.80
	Conowingo						
225	Silt loam	5.74	2.30	59.90	1.74	0.28	83.90
228	Silt loam	7.93	3.91	50 70	1 86	0 45	75.80
303	Silt loam	6.65	1.77	73 40	0.79	0.24	69 60
	Iredell						
300	Silt loam	7.96	2.76	65.40	1.36	0.27	80.20
304	Silt loam	5 64	2.34	58 50	1.04	0 30	71 20
	Lehigh						
230	Loam	6 81	2 66	60.90	1 72	0.39	77.30
231	Loam	6 17	3.69	40.20	1.91	0.52	72 80
	Manor						
95	Loam	3.20	2.87	10.30	1.88	1.05	44.10
96	Loam	4 30	2 69	37.40	1.31	0 48	63.40
97	Loam	4.33	2.88	35.80	1.31	0 21	84.00
98	Loam	4.95	2.45	50.50	1.64	0.54	67.10
99	Loam	3.88	2 97	23 40	1.72	0 52	69.80
100	Loam	3 48	2 19	37.10	1.85	0 54	70 80
101	Loam	3.78	2 98	21.40	1.55	0.43	72.20
102	Loam	4.45	2.67	40.00	1.19	0 19	84.00
103	Loam	5.55	3.12	25.80	1.09	0.26	76.20
104	Loam	3.21	3.19	0 02	0.96	0 14	85.40
105	Loam	2.64	2.07	21.60	1.48	0 19	87.20
106	Loam	2.79	2.00	28.30	1.47	0 10	92.50
107	Loam	4.23	3.31	21.70	1.43	0.16	88.80

TABLE 2—*Concluded*

SOIL NO.	SOIL TYPE	TOTAL EXCHANGE CAPACITY			ORGANIC MATTER		
		Unoxi-dized	Oxidized	Loss	Unoxi-dized	Oxidized	Loss
		m.e.	*m.e.*	*per cent*	*per cent*	*per cent*	*per cent*
	Manor (cont'd)						
108	Loam	3.53	3.19	9.60	1.43	0.43	70.00
109	Loam	3.87	3.23	16.50	1.59	0.31	80.50
110	Loam	2.72	2.69	0.03	1.29	0.28	78.30
111	Loam	4.04	2.13	47.30	1.26	0.36	71.40
112	Loam	3.50	3.29	6 00	1 85	0 72	61.10
113	Loam	2.85	2.64	7 40	1.33	0.54	59.40
114	Loam	3.25	3 02	6 50	1.24	0 64	60.00
115	Loam	3.78	2 43	35.70	1.50	0.59	60 60
116	Loam	3.21	2.35	26 80	1 60	0 64	60.00
117	Loam	3.05	3.00	1.60	1.71	0 76	55.60
118	Loam	2 84	2.50	12.00	1.67	0 33	80.20
119	Loam	3.30	2.71	17 90	1 64	0 59	64 00
120	Loam	3 82	2 32	39 30	1 79	0.45	74 90
121	Loam	4.68	2.15	54 00	1.35	0.92	31.80
122	Loam	3.98	2.52	36 70	1.40	0.74	47.20
123	Loam	3.91	2.43	12 30	1 54	0.50	67.50
124	Gravelly loam	4 35	2.53	41 60	1.69	0 21	87 60
125	Gravelly loam	4.75	3.28	52 00	1.95	0 41	79 00
126	Gravelly loam	4.50	2 95	34 40	1 21	0 10	91 70
127	Gravelly loam	3.51	2.45	30 20	1 60	0 16	90.00
128	Gravelly loam	4 20	3 08	26 60	1 62	0.22	86.40
129	Gravelly loam	2 83	2 57	9 20	1.64	0 21	87.20
130	Gravelly loam	3 22	2 77	14 00	1 86	0 19	89.80
131	Gravelly loam	3.15	2 95	6 30	1 96	0 84	57 10
132	Gravelly loam	3.31	2.94	8 20	1.96	0 69	64.80
133	Gravelly loam	4.21	3 84	8 80	1.48	0 52	64.90
134	Gravelly loam	4.42	4.14	6 30	1.84	0 47	74.40
135	Gravelly loam	4 69	3 88	17 30	1.65	1 24	24.90
136	Gravelly loam	4.77	4.29	10 60	1.72	0 64	62.80
137	Gravelly loam	5 37	3 70	31.10	1 48	0 74	50.00
	Mecklenberg						
298	Loam	6 61	4.05	38 70	1 27	0.23	81.90
299	Loam	8 90	4 50	49 40	2 09	0 36	82.80
	Montalto						
226	Clay loam	8 24	3.86	53.10	1.85	0 50	74.1
224	Clay loam	10 00	2.85	71 50	1 56	0 39	75.00
227	Clay loam	12.37	2.80	51.50	2 10	0.42	80.00
	Penn						
233	Loam	5.75	3.54	38.40	1.13	0 37	67.20
235	Loam	7.62	2.35	69.20	0.92	0.31	66 30
234	Silt loam	9.80	3.03	69.10	1.18	0.18	84.80
278	Silt loam	9.96	5.99	39.90	1.14	0.17	85.10
281	Silt loam	8.28	2.14	74.10	0.92	0.22	76.10
283	Silt loam	7.73	3.52	54.40	1.10	0.23	79.20
295	Silt loam	9.50	3.04	68.00	0.92	0.19	79.40

to texture changes for each series. Since there was considerable variation in the results among the different series and classes, the data are averaged and presented both by class and series, as well as for the whole province, in tables 6–10. Thus, table 6 is a summary of table 1, 7 of 2, 8 of 3, 9 of 4 and 10 of

TABLE 3

The influence of oxidation on the total exchange capacity and organic matter content of Appalachian Mountain and Plateau soils

SOIL NO.	SOIL TYPE	TOTAL EXCHANGE CAPACITY			ORGANIC MATTER		
		Unoxi-dized	Oxidized	Loss	Unoxi-dized	Oxidized	Loss
		m.e.	*m.e.*	*per cent*	*per cent*	*per cent*	*per cent*
	Berks						
271	Shale loam	6.21	4 39	29 3	0 95	0 44	53 7
272	Shale loam	7 88	4.72	40 2	1 27	0 97	23 6
274	Shale loam	3.70	1 93	47 8	1 23	0 35	71 6
275	Shale loam	5 45	3 17	41 4	1 40	0.50	64 3
	Dekalb						
254	Silt loam	5 96	3 20	53 7	2 50	1 23	50 8
256	Silt loam	6 15	5 40	12 2	1 17	0 56	52 1
263	Silt loam	6 95	5 41	22 2	1 95	0 79	59 5
247	Stony silt loam	5 64	6 16		2 62	1 13	56 8
255	Gravelly silt loam	5 98	5 03	15 9	0 99	0 39	60 6
260	Gravelly silt loam	5 95	5 62	5 5	1 07	0 70	34 6
262	Gravelly silt loam	3 72	1 94	47 8	1.06	0 45	57.5
294	Stony loam	6 90	5 13	25 7	2 68	1 06	60 5
	Meigs						
248	Silt loam	7 67	5 09	33 6	1 64	0.43	73 7
257	Silt loam	7 45	5 23	29 8	1 12	0 54	51 8
258	Gravelly loam	4 25	1 95	54 2	0 98	0 53	45 9
261	Gravelly loam	2 98	2 16	17 5	1 05	0 50	52 4
	Porters						
288	Silt loam	5.84	2 49	85 0	0.98	0 38	61 2
289	Silt loam	5 34	1 46	72 7	0 79	0 16	79 8
	Upshur						
249	Silt loam	6 26	3 21	48 9	1 03	0 32	70 0
253	Silt loam	5 48	4 47	18 4	2 30	1 30	43 5
250	Stony silt loam	7 10	3 92	44 3	2 33	1 25	46 5
252	Stony silt loam	4 06	2 69	33 7	2 21	0 36	83.6
268	Gravelly loam	3 69	2 95	20 3	0 62	0 49	21 0
269	Gravelly loam	2.53	2 28	9 9	0 34	0 25	26 5

5. All the values for total exchange capacity are expressed as milligram equivalents per 100 gm. of the original, untreated soils. The organic matter values are listed as per cent in terms of the original unoxidized soil. The per cent losses in exchange capacity and organic matter are calculated on the basis that the amount of each found in the untreated soil is 100 per cent.

TABLE 4

The influence of oxidation on the total exchange capacity and the organic matter content of the Limestone Valley and Upland soils

SOIL NO.	SOIL TYPE	TOTAL EXCHANGE CAPACITY			ORGANIC MATTER		
		Unoxi-dized	Oxidized	Loss	Unoxi-dized	Oxidized	Loss
		m.e.	*m.e.*	*per cent*	*per cent*	*per cent*	*per cent*
	Hagerstown						
64	Clay loam	8.81	5.14	41.6	3.14	2.02	35.7
65	Clay loam	4.26	1.80	57.7	1.98	0.54	72.8
66	Silt loam	7.67	6.32	17.6	1.34	1.15	14.3
67	Silt loam	5.22	5.01	4.0	1 72	0.66	61.6
68	Silt loam	6.84	3.06	56.9	3.26	1.03	68.4
69	Silt loam	7.84	3.25	58 5	2.60	0.81	68 8
70	Silt loam	6.18	2.20	64.4	2 70	1.24	54 1
71	Silt loam	4.05	2.19	35.9	2 56	0 36	85.9
72	Silt loam	4.84	2.56	47.1	2.24	1.86	17.1
73	Silt loam	7.23	2.20	69 6	4 28	1.14	73.4
74	Silt loam	6 70	3.33	50.3	3.26	1.86	43.0
75	Silt loam	6 82	3.56	46.2	2 79	1.40	49.8
76	Silt loam	6.12	2.29	63.2	1.93	0 86	55.4
77	Silt loam	6.29	1.80	71 4	1 24	0.95	23.4
78	Silt loam	3 84	2 33	39 3	2 24	1.03	54.1
79	Silt loam	2.93	2.08	29 0	1.42	1.05	26.1
80	Silt loam	3.24	2 57	20.7	2.00	1.09	45.5
81	Silt loam	2.75	2.74	0 4	2.22	1.12	49.5
82	Silt loam	6.01	4.36	26.0	1.36	0 50	63.2
83	Silt loam	4.41	2 03	54.1	1.83	0.64	65.0
84	Silt loam	5.52	2 47	55.3	1.62	0.57	64.8
	Frankstown						
48	Silt loam	8 02	4 89	39.0	1.24	0.57	54.1
49	Silt loam	6.44	3.73	42.1	1.40	0.79	43.6
50	Silt loam	4 90	2.76	43.7	1.17	0.76	35.1
51	Silt loam	4.19	3 12	25 5	1.17	0.95	18.8
52	Silt loam	6.05	4.03	33 4	1.31	0.40	69.5
53	Silt loam	6.76	5.71	15 5	1.57	0.60	61.8
54	Silt loam	4.88	3.16	35.2	2.00	0.59	70.5
55	Silt loam	3.71	3.32	10.5	2.18	0.71	67.4
56	Silt loam	4.06	3.71	8.6	2.16	0.45	79.2
57	Silt loam	5.01	3.77	24.8	1.72	0.60	65.1
58	Silt loam	3.95	2.62	33.7	2.09	0.33	84.2
59	Silt loam	3.16	3.00	5.1	2.04	0.17	91.7
60	Silt loam	4.60	1.41	69.3	2.12	0.43	79.7
61	Silt loam	2.84	2.69	7.0	1.38	0.16	88.4
62	Silt loam	4.19	3.37	19.6	1.26	0.16	87.3
63	Silt loam	2.57	2.51	2.3	1 48	0.16	89.2
	Murrill						
276	Gravelly loam	2 40	2.06	14.2	0.72	0.20	72.2
286	Gravelly loam	5 24	2.62	50.0	1.64	0.29	82.3
	Westmoreland						
266	Silt loam	1.95	3.74		1.14	0.32	71.9
267	Silt loam	2.10	3.82		0.67	0.25	62.2

DISCUSSION

There was considerable variation in the influence of organic matter removal on the total exchange capacity of individual soils. This is shown by the data in tables 1–5. The variations occurred not only among the various series but also within the classes of the series. These differences were ascribed to the removal of the organic matter, although it was probably influenced by one or more of the following factors: cultural practices, parent material, soil texture, composition of the organic matter, number of samples, error in

TABLE 5

The influence of oxidation on the total exchange capacity and organic matter content of the River Terrace and Flood Plain soils

SOIL NO.	SOIL TYPE	TOTAL EXCHANGE CAPACITY			ORGANIC MATTER		
		Unoxi-dized	Oxidized	Loss	Unoxi-dized	Oxidized	Loss
		m.e.	*m.e.*	*per cent*	*per cent*	*per cent*	*per cent*
	Atkins						
251	Silty clay loam	9 92	4 87	51.0	1 95	1 30	33.3
259	Silty clay loam	9 86	5 86	40 6	1 80	0 74	58 9
	Bermudian						
279	Silt loam	2 60	1.47	43.5	1 30	0 27	79.2
280	Silt loam	5 60	4 96	8 9	1.12	0 22	80 3
284	Silt loam	5 95	5 42	13 4	1 33	0 61	54 1
	Congaree						
232	Silt loam	7 30	4 63	36 8	2 43	0 74	69 5
277	Silt loam	4 86	3.63	11 2	1 64	0 29	82 3
302	Silt loam	4 84	1.49	69 3	2 71	1.29	57 4
305	Silt loam	6 31	2 58	59 1	1 40	0 19	86 4
	Holston						
273	Silt loam	3 54	1 96	44 6	1.21	0.29	76 0
	Huntington						
270	Silt loam	4 53	2 72	40.0	1 33	0 59	55 6
301	Silt loam	6 17	3.76	39.1	2 52	1.78	29 4
264	Loam	4.66	2 98	36 0	1 11	0 53	52.3
	Wehadkee						
229	Silt loam	2 90	2 16	25 5	1.37	0.56	59 1
287	Silt loam	6 24	5 92	5.1	1.51	0.42	72 3

sampling, incomplete oxidation of organic matter, an increase in the mineral exchange complex, and a greater dispersion by the hydrogen peroxide treatment. Undoubtedly some of these influencing agencies increased, and others decreased, the importance of organic matter in the exchange complex. There is a possibility that many of the influencing factors counteracted one another. It is believed, regardless of these possible errors, that the data obtained in the study show the importance of organic matter in the exchange complex.

The data for the Coastal Plain soils, as given in table 1, indicate considerable

TABLE 6

Summary for Coastal Plain soils, showing the influence of oxidation on total exchange capacity and organic matter content by class and series

SOIL TYPE	NUMBER OF SAMPLES	TOTAL EXCHANGE CAPACITY			ORGANIC MATTER		
		Unoxidized	Oxidized	Loss	Unoxidized	Oxidized	Loss
		m.e.	m.e.	per cent	m.e.	m.e.	per cent
CLASS:							
Silt loams							
Elkton	10	3.42	2 16	36 9	1 27	0 25	80 3
Keyport..	6	3.89	2.29	41.1	1.39	0.24	81 7
Leonardtown	6	3.22	1.87	41.9	1.10	0.23	79 1
Sassafras..	21	2.88	1.87	35 1	1.47	0 21	93 0
Average	43	3.19	2 00	37.3	1.66	0.23	84 2
Loams							
Elkton.	2	2.87	1.16	59 6	0.84	0.08	90.5
Keyport.........................	2	3.15	0.95	69.8	1.24	0 08	93.6
Portsmouth..................... ..	4	21 36	5.14	76 0	5.29	1 45	72.6
Sassafras.......................	8	2 13	0.90	57 8	0.98	0.13	86 7
Average, for all....	16	7 16	2.00	72.1	2.08	0 45	78 5
Average, all except Portsmouth .	12	2 44	0 95	64 2	1.00	0 11	88 5
Fine sandy loams							
Collington*..	6	4.40	3.08	30.0	0.84	0.14	83 1
Keyport.	4	2 80	1.14	59 3	1 05	0 23	78 1
Portsmouth	3	11 43	1 15	90.0	3.11	0 59	81.0
Average, for all	13	5.53	1 13	76.6	1 42	0 27	81 0
Average, all except Portsmouth...	10	3 76	2 30	41.7	0 92	0 17	81 1
Sandy loams							
Collington........	1	2 14	1.29	39.8	0 52	0 24	54 7
Sassafras.......................	4	1 87	0.69	63.2	0 69	0 14	79.7
Average.....	5	1 92	0.81	57 8	0 66	0 19	71 2
Loamy sands							
Sassafras	4	1 94	0.84	56.7	0.47	0 10	79 3
SERIES:							
Collington	7	4 08	2.82	30.9	0.79	0 15	80.3
Elkton.........................	12	3 33	1 99	40 0	1.20	0 22	81 7
Keyport....	12	3 40	1 79	47 4	1 25	0 21	83.2
Leonardtown......	6	3 22	1 87	35.1	1.47	0 21	93.0
Portsmouth........	7	17 10	3 43	80 0	4 36	1 08	75 2
Sassafras....................	37	2 51	1.42	43 3	1.17	0 17	85.3
All types.......	81	4 21	1 99	53 2	1.45	0 26	81 9
All types, except Portsmouth........	74	2 99	1.74	41.9	1 18	0.18	84 2

* This includes the two Collington very fine sandy loam samples.

variation in the two forms of the exchange complex.   The soils with the less maturely developed profiles had the greatest difference between individual samples.   This could be the result of unavoidable errors in sampling.   The results for the mature soils, such as the Sassafras and Leonardtown, were the most uniform.   This consistency can probably be attributed to several causes,

TABLE 7

Summary for the Piedmont soils, showing the influence of oxidation on total exchange capacity and organic matter content by class and series

SOIL TYPE	NUMBER OF SAMPLES	TOTAL EXCHANGE CAPACITY			ORGANIC MATTER		
		Unoxi-dized	Oxidized	Loss	Unoxi-dized	Oxidized	Loss
		m.e.	*m.e.*	*per cent*	*per cent*	*per cent*	*per cent*
CLASS:							
Loams							
Ashe.	2	5 85	3 66	37.5	1 02	0 27	73 5
Chester . . .	10	4 51	3 01	33 3	1.76	0 41	76 7
Lehigh. . . .	2	6 49	3 18	51 0	1.82	0 46	74.8
Manor. . . .	29	3 76	2 72	27 6	1.48	0 47	68 2
Mecklenberg.	2	7 76	4 28	44 8	1 68	0 30	82.1
Penn	2	6 68	2.94	55 9	1.02	0 34	66.6
Average.	47	4 42	2 92	34 1	1 52	0 44	71.4
Gravelly loam							
Ashe	2	8.29	4 22	49 2	1.13	0 35	69 0
Manor	14	4 02	3 24	19 5	1 69	0 57	66.2
Average .	16	4 55	3 36	26.1	1 62	0 54	66 5
Slate loam							
Cardiff. .	2	7 67	2 77	63.6	1 47	0 24	83 7
Stony loam							
Ashe	1	5 85	3 66	37 5	1 02	0 27	73 5
Silt loam							
Conowingo	3	6 77	2 66	60 7	1 46	0.32	84.2
Iredell	2	6 80	2 55	62 5	1 20	0 28	76 7
Penn	5	9 05	3 54	60 8	1 05	0 20	81 0
Average.	10	7 92	3 08	61 2	1 20	0 25	79 2
Clay loam							
Montalto .	3	10 20	3 17	69 0	1 83	0 44	79 9
SERIES:							
Ashe	5	6 83	3.88	43 2	1 06	0.26	75 5
Cardiff	2	7.61	2.77	63.6	1 47	0 24	83 7
Chester . . .	10	4 51	3 01	33 3	1.76	0 41	76 7
Conowingo . .	3	6 77	2.66	60 7	1 46	0 32	84 2
Iredell	2	6.80	2 55	62 5	1 20	0 28	76 7
Lehigh .	2	6 49	3 18	51 0	1 82	0 46	74 8
Manor	43	3.84	2 89	24.8	1 55	0.50	67 8
Mecklenberg.	2	7 76	4 28	44 8	1 68	0 46	74 8
Montalto	3	10 20	3 17	69 0	1.83	0 44	79 7
Penn	7	8 37	3 37	59.7	1.04	0 24	67 0
Piedmont average.	79	5 21	3 04	41.6	1 51	0 44	70 9

but the stage which the organic matter reached in decomposition may be a very important consideration. When the results on these soils are arranged by class regardless of series, as in table 6, the texture exerts considerable influence. The coarser textured soils (more sandy) contained less organic matter, which was less completely oxidized. On the other hand, these coarser soils

lost a greater percentage of their exchange complex by oxidation. This indicates that the organic matter in the sandy soils may be more active in the exchange complex. The value of organic matter in sandy soils has been emphasized for a long time, and these results indicate that such emphasis has not been overdone. The general averages, as given in table 6, are calculated with and without the Portsmouth soils. This was done in order to prevent these soils, with their large content of organic matter and lack of maturity, from having too great an influence on the results from the other soils of this province. Since the Coastal Plain soils are generally coarser in texture than the soils of the other provinces in the state, the value of organic matter in helping to maintain these soils in a high state of potential fertility cannot be overlooked.

The data, as given in table 2, for the Piedmont soils show considerable difference in the organic and total exchange capacity of these soils. The extremes of this diversity are not so large as they were in the Coastal Plain soils. On the whole, the Piedmont samples had a higher exchange capacity and organic matter content. This increase in exchange complex seemed to be largely in the inorganic fraction, since the loss by oxidation was practically the same as for the Coastal Plain soils. If the organic matter had been as completely removed as it was in the Coastal Plain soils, the loss in the exchange capacity of the Piedmont soils would probably have been greater. The loss in exchange capacity in many of the Piedmont soils was greater than the total exchange capacity of several of the Coastal Plain soils. Since the texture of the Piedmont soils is more uniform than that of the Coastal Plain soils, the influence of particle size, as shown by the data in table 7, is not so great. Both the inorganic and organic exchange capacities increased with the greater division of the soil particles. Although the Piedmont soils varied considerably in their parent material, the total exchange capacity was similar. There was considerable variation, however, when the amount of organic exchange complex was compared. The soils from the sedimentary rocks, such as the Penn, Lehigh, and Cardiff soils, had a greater portion of their total exchange capacity in the organic form than the igneous and granite soils, such as Ashe and Chester. As the soil became more mature, the organic complex increased. The Ashe soils, although belonging to the Appalachian Mountain and Plateau province, are grouped with the Piedmont soils, as they are similar in appearance, parent material, crop response, and use to the Chester soils. Although many of the Piedmont soils had a large amount of organic matter in the exchange complex, the extensiveness of the Ashe, Chester, and Manor soils reduced this value for this province considerably.

The results for the Appalachian Mountain and Plateau soils, as given in table 3, vary widely in the total exchange capacity, organic exchange complex, and amount of organic matter. This diversity is, to a certain extent, according to the texture and series. The data given in table 8, however, indicate that the variations according to the texture and class are small. This should

be expected, since the actual texture of the soils, after the stone, gravel, and slate have been removed, is very similar. The amount of organic matter destroyed by hydrogen peroxide, with the exception of Porters soil, was less than for either of the previously discussed provinces. Although it is of little concern in this paper, the data indicate that the climatic conditions under which these soils have developed have influenced the decomposition of the

TABLE 8

Summary for the Appalachian Mountain and Plateau soils, showing the influence of oxidation on the total exchange capacity and organic matter content by class and series

SOIL TYPE	NUMBER OF SAMPLES	TOTAL EXCHANGE CAPACITY			ORGANIC MATTER		
		Unoxidized	Oxidized	Loss	Unoxidized	Oxidized	Loss
		m.e.	*m.e.*	*per cent*	*per cent*	*per cent*	*per cent*
CLASS:							
Silt loam							
Dekalb	3	6 35	3 67	26 9	1 87	0 86	54 1
Meigs	2	7.56	5 16	31 7	1 38	0 48	62.8
Porters	2	5 59	1 98	78 9	0 89	0 27	70 5
Upshur	2	5.87	3 84	33 7	1 67	0 81	56.8
Average	9	6 34	4 00	37 1	1 50	0 63	57 6
Gravelly silt loam							
Dekalb	3	5 25	4 20	23.1	1 04	0 51	50 9
Stony silt loam							
Dekalb	1	5.64	6.16	....	2 62	1 13	56 8
Upshur	2	5 58	3 31	39 0	2 72	0 81	65 1
Average	3	5 60	4.26	24 0	2 39	0 91	61 8
Gravelly loam							
Meigs	2	3 62	2 06	35 9	1 02	0 52	49 2
Upshur	2	3 11	2 62	15 1	0 48	0 37	23 8
Average	4	3 36	2 33	20 6	0 57	0 44	40 8
Shale loam							
Berks	4	5 81	3 55	39 7	1 21	0 57	53 3
Stony loam							
Dekalb	1	6 90	5 13	25 7	2 68	1 06	60 5
SERIES:							
Berks	4	5.81	3 55	39 7	1 21	0 57	53.3
Dekalb	8	5 90	4 74	19 8	1 78	0 79	55.1
Meigs	4	5.60	3 86	35 4	1.20	0 50	58 3
Porters	2	5 59	1.98	78 9	0 89	0 27	70 5
Upshur	6	4 85	3 51	33 0	1 47	0 66	55 2
Average	24	5.55	3 75	32 4	1 43	0 63	56.0

organic matter, as it seems more resistant to hydrogen peroxide treatments. The Porters soils contained a large amount of the exchange complex in the organic form. This is probably the result of a more complete oxidation of the organic materials. The poorest soils in this province were affected the least by the hydrogen peroxide treatment. The Appalachian Mountain and Plateau soils seemed to contain less organic matter in the exchange complex.

The individual variations in exchange capacity and organic matter content of the samples from the Limestone Valley and Upland province were large. Even after oxidation there was considerable difference for the individual samples. The data, given in table 4, show also that the hydrogen peroxide treatment increased the inorganic exchange capacity in some of the soil. The general average, given in table 9, indicates that these variations continue in the series and classes. The finer textured soils in this province contained more organic matter as well as a larger organic exchange complex. Since this could not be the result of a more complete oxidation, the organic fraction must have a higher sorbtion capacity in the finer soils. On the whole, the

TABLE 9

Summary for Limestone Valley and Upland soils, showing the influence of oxidation on total exchange capacity and organic matter content by class and series

SOIL TYPE	NUMBER OF SAMPLES	TOTAL EXCHANGE CAPACITY			ORGANIC MATTER		
		Unoxi-dized	Oxidized	Loss	Unoxi-dized	Oxidized	Loss
		m.e.	*m.e.*	*per cent*	*per cent*	*per cent*	*per cent*
CLASS:							
Clay loam							
Hagerstown..............	2	6 54	3 46	47.2	2 56	1.28	50.0
Silt loam							
Frankstown..............	16	4.71	3 36	28.6	1 64	0.49	70.2
Hagerstown..............	19	5 50	2 97	46 1	2.24	1.02	54.7
Westmoreland	2	2 03	3 78		0 91	0.29	68.0
Average..........	37	4 97	3 18	36 0	1.91	0.75	60.8
Gravelly loam							
Murrill..................	2	3.82	2 34	38.7	1.18	0.25	79.2
SERIES:							
Frankstown..............	16	4 71	3.36	28.6	1.64	0.49	70.2
Hagerstown	21	5 60	3 01	46 2	2 27	1.04	54.2
Murrill............	2	3.82	2 34	38 7	1.18	0 25	79 2
Westmoreland	2	2 03	3 74		0.91	0 29	68.0
Average...............	41	4 99	3 15	36 8	1.91	0.75	60.7

Limestone soils compared favorably with the Piedmont and Mountain soils with which they are associated.

The River Terrace and Flood Plain soils, as shown by the data in table 5, were also variable in their organic and inorganic exchange complex and in the amount of organic matter. This is more or less to be expected, since they are formed by the wash from the higher soils and vary widely in their parent material, texture, organic matter, and drainage. The small number of samples from these soils may also account for this variation. These soils resemble the Limestone soils, as shown by the data in table 10, in that the finer textured soils had the highest exchange complex and the greatest loss by oxidation. The Atkins soil was much higher in its exchange capacity than any

of the other soils. This is probably due to its very fine texture. The difference in results obtained for the individual series probably can be attributed to the variation in the material from which these soils are formed. For example, those formed from surface wash of the sandstone soils were much lower in their exchange capacity than those from granitic soil. The averaged data for each series in this group resemble the values for the respective soils in the Limestone, Piedmont, and Mountain provinces from which they were derived.

TABLE 10

Summary for River Terrace and Flood Plain soils, showing the influence of oxidation on total exchange capacity and organic matter content by class and series

SOIL TYPE	NUMBER OF SAMPLES	TOTAL EXCHANGE CAPACITY			ORGANIC MATTER		
		Unoxidized	Oxidized	Loss	Unoxidized	Oxidized	Loss
		m.e.	*m.e.*	*per cent*	*per cent*	*per cent*	*per cent*
CLASS:							
Silt loam							
Bermudian............. ..	3	4 72	3 95	16 3	1 25	0 37	70 7
Congaree..	4	5 83	3 08	47 3	2 05	0 63	69.3
Holston. . .	1	3 54	1 96	44 6	1 21	0 29	76 0
Huntington.	2	5 80	3 24	44 1	1 93	1 19	38 4
Wehadkee.	2	4 57	4 04	11 6	1 44	0 49	66 0
Average . . .	12	5 15	3 39	34 1	1 66	0 60	63 5
Loam							
Huntington . . .	1	4 66	2 98	36 1	1 11	0 53	52 3
Silty clay loam							
Atkins . .	2	9 89	5 37	45 8	1 88	1 02	45 6
SERIES:							
Atkins	2	9 89	5 37	45.8	1 88	1 02	45 6
Bermudian	3	4.72	3 95	16 3	1 25	0 37	70 7
Congaree	4	5.83	3 08	47 3	2 05	0 63	69 3
Holston	1	3 54	1.96	44 6	1 21	0 29	76 0
Huntington	3	5 42	3 15	41 9	1 65	0 97	41 5
Wehadkee	2	4 57	4 04	11 6	1 44	0 49	66 0
Average	15	5 75	3 63	36 9	1 65	0 65	60 2

A statistical analysis[2] of the data obtained in this study was made. Coefficients of correlation for many factors were obtained for the soils, by provinces. This preliminary analysis indicated that a comparison between the percentage of organic matter loss by oxidation and the loss in exchange capacity by this treatment might perhaps be significant. Accordingly, the coefficient of correlation between these two factors was calculated separately for the soils of each series. Since the limited number of samples from each series caused the odds of these correlations to be very low, these values were aver-

aged by provinces.   This was done by converting to z values and correcting for small numbers.   The significance of each averaged z value was then determined.   Here again, the number of observations in any one province was too small in comparison with the size of the correlation to permit statistical significance to be ascribed to it.   When the averaged z value for all provinces was obtained, however, it was highly significant (odds approximately 99 to 1), even though the coefficient of correlation conforming to this z value is only $+.23$ in size.   This does not prove, but indicates, that had the number of observations in each series been large or the variation between individual samples small, the individual coefficients of correlation would probably have been significant.   This correlation gives support to the belief that these data show organic matter to be very valuable in maintaining or increasing the fertility of Maryland soils.

SUMMARY

The purpose of this study was to estimate the amount of organic matter in the exchange complex of Maryland soils.   The total exchange capacity was obtained by leaching with ammonium acetate and measuring the amount of ammonia absorbed.   The organic matter was oxidized with 6 per cent hydrogen peroxide.   Total carbon determinations were made to measure the amount of organic matter in each soil before and after oxidation.   Both the exchange capacity and organic matter were estimated before and after the treatment with hydrogen peroxide.   The soils studied and results obtained were grouped according to their series and class within the five soil provinces.   Many factors doubtlessly affected the data; however, the general trend justified the drawing of definite conclusions from the results.

The Coastal Plain soils had the largest percentage of organic matter which was active in the exchange complex.   The coarser or sandy classes in this group had the highest percentage of the total exchange capacity in the organic form.   The percentage of organic matter taking part in the total exchange capacity was practically the same in the Piedmont and Coastal Plain soils.   The finer textured soils in all the provinces, except the Coastal Plain soils, showed the greatest loss in exchange capacity by oxidation.   The average value of the organic exchange complex obtained or, conversely, the amount of the complex destroyed by oxidation, was similar for the Appalachian and Mountain and Plateau, the Limestone Valleys and Uplands, and the River Terrace and Flood Plain soils.   A statistical study of all the values obtained for the loss in organic matter and the loss in exchange capacity for all the soils as a unit showed a highly significant correlation.

REFERENCES

(1) HECK, A. F.   1929   A method for the determination of total carbon and also for the estimation of carbon dioxide evolved from the soil.   *Soil Sci.* 28: 225–233.

(2) JOHNSON, A. H., AND GREEN, J. H.   1930   Modified methyl red and sodium alizarin sulfonate indicators.   *Indus. and Engin. Chem. Anal. Ed.* 2: 2–4.

(3) SCHOLLENBERGER, C. J., AND DREIBELBIS, F. R.   1930   Analytical methods in base exchange investigations on soils.   *Soil Sci.* 30: 161–173.

CHANGES IN ORGANIC MATTER IN WESTERN WASHINGTON SOILS AS A RESULT OF CROPPING[1]

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The current conception of the effect of cropping on the organic matter of the soil is that considerable loss occurs under general farming systems. The basis of this idea is obtained from the work of many investigators (1, 6, 7), and the conclusions are no doubt justified. The investigations to be reported in this paper indicate, however, that it is not safe to conclude that soils everywhere behave similarly under agricultural use.

COLLECTION OF SOIL SAMPLES

In an attempt to obtain a basis for an agricultural rating of several soil types in western Washington for purposes of land classification, a number of paired samples of soil were obtained for analysis. It seemed desirable to find out what changes the different soil types underwent in agricultural use. Historical records with respect to type of farming, length of time under cultivation, and kind of management were obtained from the owner of each field where samples were taken. Locations were chosen so that closely adjacent areas of uncropped soil were available. Samples were taken to the same depth from both cropped and uncropped soils, and this depth was determined by observing the thickness of the plowed soil in cultivated fields. In all cases the uncropped soils had been subjected to the compulsory slash burning which follows lumbering operations. Although most of the original forest floor was thus destroyed, the elapse of several years since the burning has tended to restore the surface accumulations, particularly in the second growth areas. Slash burning does not usually affect the mineral surface soil except by the addition of ashy residues and charcoal. The soil is not heated so that volatilization of its own organic matter takes place. Although these samples were not truly virgin soils, nevertheless they were representative of the conditions in each cultivated soil at the time it was originally cleared for cropping.

Soil samples obtained in cultivated fields and in adjacent uncropped areas were not considered comparable unless they were found to possess a marked

139

similarity in textural character. Seventy-three pairs of such samples, representing seven soil series, were obtained in the autumn of 1934 in one county in western Washington, and analyses were made for texture, reaction, quantity, and quality of organic matter.

Samples were air-dried, passed through a 2-mm. screen with round holes, mixed, and stored in air-tight containers.

Mechanical analysis

The Bouyoucos (4) hydrometer method was used for this work, and separations of the sands as a group, the silt, and the clay fractions were made. The organic matter was not removed and, since all samples were free from carbonates, no acid treatments were made.

Reaction

Determinations of pH values were made, using the quinhydrone method. Water extracts of the ratio—1 part of soil to 5 parts of boiled distilled water—were allowed to stand 1 hour with frequent shaking before measurements were made. Unusual drifts in potential were not troublesome in these soils.

Organic matter

The titration method of Walkley and Black (8) was used in these determinations, and the soils were ground to pass a 100-mesh screen before analysis. The values for organic carbon were multiplied by the factor 1.724 to obtain values for organic matter. A check on the suitability of the titration method was made, using the official wet combustion method.

Total nitrogen

The standard Kjeldahl-Gunning method was employed with soil samples which had been ground to pass a 100-mesh screen.

Nitrification

Soils which had passed the 2-mm. screen were saturated with 5 times their weight of distilled water, shaken once, and allowed to stand 15 minutes. The entire mass was thrown on a large Büchner filter, covered, and allowed to drain with suction, according to the method Bouyoucos (3) suggests for the rapid determination of the moisture equivalent. One hundred grams of the soil was then thoroughly mixed in oilcloth and placed in cotton-plugged Erlenmeyer flasks for incubation at 25°C. At suitable intervals samples were removed, dried, extracted with water, and analyzed for nitrates according to the phenoldisulfonic acid method. The values for original nitrate content were obtained by analysis of the original filtrate. All soils, regardless of tex-

tural differences, were incubated by this method at the same relative moisture content.   The results were calculated to show the concentration in the soil.

TYPE OF SOIL DEVELOPMENT IN THE REGION

As a result of extensive regional studies, Nikiforoff (5) states that these soils belong to a group of yellow-brown soils with certain unique characteristics.   They have developed under a forest vegetation, mainly coniferous, in a maritime climate noted for its relatively mild wet winters and dry summers with moderate temperatures.   More detailed description of the forest cover has been given elsewhere (9).   The climatic conditions in the region where the samples were obtained may be best summarized in the form of a table compiled from official records of the Weather Bureau (table 1).   The annual average relative humidity in western Washington is 70.

TABLE 1

Climatic conditions within the sampling area

LOCATION	AVERAGE RAINFALL												
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Annual
	in.	in.	in.	in.	in.	in.	in.	in.	in.	in.	in.	in.	in.
Everett ..	4 50	3 20	3 33	2 52	1 93	1 49	0 70	1 06	1 98	3 29	3 70	4 81	32 51
Snohomish	5 21	4 69	4 00	3 39	3 06	2 44	1 28	1 11	2 65	3 54	6 20	5 95	43.52
Silvana	4 47	4 84	3 40	3 20	3 27	1 77	0 78	1 04	1 80	2 38	4 77	4 06	35 78
Granite Falls ..	7 14	5 16	5.87	4.95	4 28	3 58	1 68	1 75	3 78	5 70	7 66	7 86	59 41

	MEAN TEMPERATURE												
	de-grees F.	de-grees F.	de-grees F.	de-grees F.	de-grees F.	de-grees F	de-grees F	de-grees F.	de-grees F.	de-grees F.	de-grees F.	de-grees F.	degrees F.
Everett . .	37 2	40 4	43 8	48 4	53 2	58 2	61 3	61 0	56 2	50 3	44.2	38 7	49.4
Snohomish . .	37 8	40 6	44 0	49 4	54 6	59 2	61 6	62 5	57 6	51 3	44 4	39 8	50 7

EXPERIMENTAL RESULTS

Reliability of the titration method for organic carbon

In view of the fact that large numbers of determinations were required, it was decided that an examination of the rapid titration method should be made by comparing results obtained with it with those obtained with the official wet combustion method.   Six pairs of soil samples were chosen, representing upland, terrace, lake bottom, and alluvial areas.   Wide differences in the quality of organic matter probably occur in these areas because of the differences in native vegetation which covered them.   The results of the comparative study are shown in table 2.

The rapid titration method appears to be satisfactory for the work of comparing cropped and uncropped soils.   Although results with individual

samples show differences as large as 0.68 per cent, the averages for 12 soils agree closely. The correction factor suggested by Walkley and Black seems to be equally applicable to cropped and virgin soils, because the results obtained by its use compare closely with the results with the wet combustion method. These findings are in agreement with those reported by Baker (2).

Quantity of organic matter

The results of the textural determinations, pH values, and organic matter for 73 pairs of soils are presented in table 3. These series represent upland soils entirely.

TABLE 2

Results of the determination of organic carbon on individual samples by the official wet combustion method and the rapid titration method of Walkley and Black

SAMPLE NUMBER	SOIL TYPE	ORGANIC MATTER BY		DIFFERENCE a — b
		(a) Wet combustion method	(b) Rapid titration method	
		per cent	*per cent*	
	Alderwood loam			
143	Cropped	5.37	5.36	+0 01
143a	Virgin	5.25	5 34	−0 09
	Satsop loam			
141	Cropped	8.28	7 87	+0.41
141a	Virgin	5.61	5 58	+0.03
	Kellogg silt loam			
133	Cropped	6.21	6.89	−0.68
133a	Virgin	5.28	5 84	−0.56
	Kellogg sandy loam			
134	Cropped	2.25	2.14	+0.11
134a	Virgin	4.23	3.98	+0 25
	Kellogg loam			
135	Cropped	3 42	3 44	−0.02
135a	Virgin	4 05	4.35	−0.30
	Puget sandy loam			
142	Cropped	3.36	3.30	+0.06
142a	Virgin	2.28	2.15	+0.13
Average—12 soils....................		4.63	4.69	−0.06

The data for the Alderwood series are the most extensive, as this is the most widely distributed upland soil in the region. Of 41 pairs of samples, 32 show definitely larger quantities of organic matter in the cropped soil, 4 pairs are nearly alike, and only 5 uncropped samples exceed the corresponding cropped samples in this constituent. It will be noted that when the common bracken fern (*Pteridium aquilinum pubescens*) was present in the cover of the un- cropped samples, a considerable build-up in organic matter occurred, which accounts for the apparent loss in organic matter through cropping in at least

TABLE 3

Comparison of cropped and uncropped soils of seven soil series in western Washington with particular reference to changes in pH values and organic matter

SAMPLE NUMBER	AGRICULTURAL HISTORY	SAND	SILT	CLAY	pH VALUE	ORGANIC MATTER	
						Amount	Change
		per cent	*per cent*	*per cent*		*per cent*	
		Alderwood series					
7	Cereals 4 yr., no fert.	66	17	17	5 7	4 67	+1.22
	Cutover	51	34	15	6 0	3 45	
9	Cereals, hay 11 yr., no fert.	55	36	9	5.9	4 28	+0 38
	Cutover	66	25	9	5.7	3.90	
14	Vineyard 6 yr., chick manure	62	30	8	6 0	6 96	+3 65
	Second growth	65	23	12	5 8	3.31	
18	Rotation, 20 yr., with manure	62	30	8	5 7	8.78	+3 76
	Stump pasture	64	27	9	5 4	5.02	
20	Oats, clover 5 yrs., no fert.	62	28	10	5 9	8 67	+1 38
	Stump pasture	62	28	10	5 7	6 29	
22	Truck crops 10 yr., heavy fert.	39	40	21	5 9	8 64	+4 66
	Cutover	37	44	19	5 7	3.98	
23	Rotation, 30 yr., with manure	53	36	11	5 9	7 81	+0 67
	Second growth	59	33	8	5 5	7.14	
26	Hay 5 yr., with manure	59	33	8	5 8	8.93	+5.09
	Second growth	63	29	8	6 1	3 84	
27	Hay 6 yr., no fert.	53	35	12	5.9	6 38	+0 35
	Stump pasture	56	29	15	5 8	6 03	
29	Garden 6 yr., with manure	62	26	12	6 4	5 02	+0 24
	Cutover	61	30	9	5.7	4 78	
31	Cropped 45 yr., recently idle	61	31	8	5 6	3 69	+2 41
	Second growth	63	30	7	5 8	1 28	
33	Vineyard 10 yr., chick and guano	60	33	7	6 2	6 28	+3 68
	Cutover	70	25	5	6 3	2 60	
34	Vineyard 5 yr., 1st yr. manure	66	25	9	6.4	3 07	− 3 79
	Cutover	65	25	10	6 2	6 86	
37	Hay 10 yr., no fert.	63	30	7	5.7	5 41	+1 39
	Stump pasture	58	30	12	5 9	4 02	
40	Rotation 20 yr., some manure	63	26	11	6 2	8 52	+2 81
	Stump pasture	66	24	10	6.1	5.71	
41	Strawberries 15 yr., chick manure	59	33	8	6.0	6.10	+1 39
	Cutover	56	36	8	5 8	4 71	
42	Clover, cereals 5 yr., no fert.	62	28	10	5.9	4 58	+0.03
	Cutover	61	29	10	6 0	4.55	
56	Rotation 30 yr., with manure	56	33	11	6.1	8 12	+3.98
	Cutover	59	32	9	6.0	4.14	
60	Rotation 40 yr., no fert.	50	37	13	6.0	5.86	− 3 52
	Cutover in ferns	50	38	12	5.9	9 38	
62	Rotation 30 yr., little fert.	55	35	10	6 2	7 41	+1 72
	Second growth	55	36	9	5 9	5 69	
66	Rotation 10 yr., heavy chick manure	63	27	10	6 1	8 71	+2 88
	Second growth	63	27	10	5 7	5.83	

TABLE 3—*Continued*

SAMPLE NUMBER	AGRICULTURAL HISTORY	SAND	SILT	CLAY	pH VALUE	ORGANIC MATTER	
						Amount	Change
	Alderwood series—*Concluded*						
		per cent	per cent	per cent		per cent	
67	Rotation 30 yr., heavy manure	50	33	17	5 6	7 36	+3.67
	Second growth	56	33	11	5 8	3 69	
68	Rotation 11 yr., no manure	52	35	13	6 0	6 43	+2 12
	Second growth	52	34	14	5 8	4 31	
69	Rotation 7 yr., no fert.	65	26	9	6.1	5 48	−0 47
	Second growth	64	27	9	5 9	5 93	
73	Orchard in grass 15 yr., no fert.	61	31	8	5.9	6.14	+3 97
	Cutover	60	33	7	6 2	2 17	
76	Rotation 15 yr., with manure	54	34	12	5 7	8 10	+4 15
	Cutover	57	31	12	5 8	3 95	
80	Rotation 14 yr., without manure	58	32	10	5 8	3 90	−0 98
	Cutover	53	36	11	5 7	4.88	
81	Rotation 25 yr., some manure	55	36	9	6 1	5 96	+0 25
	Stump pasture	56	35	9	6 2	5 71	
84	Cropped 4 yr., with chick manure	66	26	8	5 9	2 64	+1 26
	Cutover	71	21	8	5 9	1 38	
91	Rotation 8 yr., no manure	64	28	8	6.1	5 00	+1 72
	Second growth	65	28	7	6 2	3 28	
92	Orchard 8 yr., no fert.	58	33	9	5.9	6 65	−4 28
	Second growth with ferns	59	33	8	5 8	10 93	
93	Potatoes 2 yr., no fert.	53	31	16	5 7	5 81	−0 74
	Alder bottom	52	36	12	5 3	6 55	
94	Oats 2 yr., no fert.	63	28	9	6 0	7.84	−0 06
	Cutover with ferns	57	34	9	5 6	7 90	
101	Rotation 25 yr., little manure	69	23	8	6 1	2 70	+0 01
	Second growth	72	21	7	6 2	2 69	
102	Rotation 46 yr., with manure	58	33	9	6.0	5 55	+1 93
	Second growth	60	31	9	6.0	3 62	
104	Grass 8 yr., no fert.	64	29	7	6 2	7 14	+0 43
	Second growth	62	31	7	5 5	6 71	
105	Rotation 7 yr., heavy chick manure	53	37	10	5 8	8.22	+1 96
	Cutover	54	39	7	6 1	6 26	
111	Rotation 12 yr., no fert.	53	36	11	5 2	8 86	+1 28
	Second growth	52	39	9	5 5	7 58	
118	Rotation 15 yr., with manure	51	41	8	5 9	5 78	+1.71
	Cutover	53	39	8	5.9	4 07	
125	Orchard 25 yr., in grass	61	31	8	6 1	8.65	−1.14
	Cutover with ferns	60	32	8	5.6	9 79	
143	Cereals 5 yr., no fert.	56	32	12	5.8	9 24	+0.03
	Cutover with ferns	54	33	13	5.7	9 21	
Average for the series	c...	58	31	11	5 94	6.47	+1.27
	v..................	59	31	10	5.85	5.20	

TABLE 3—*Continued*

SAMPLE NUMBER	AGRICULTURAL HISTORY	SAND	SILT	CLAY	pH VALUE	ORGANIC MATTER	
						Amount	Change
	Everett series						
		per cent	*per cent*	*per cent*		*per cent*	
5	Rotation 8 yr., with alfalfa	74	19	7	6 1	6 43	+3 78
	Stump pasture	76	16	8	5 8	2 65	
6	Rotation 30 yr., with manure	57	35	8	5 7	7 48	+2 50
	Stump pasture	69	23	8	6 0	4 98	
12	Rotation 12 yr., no manure	78	17	5	6 2	6 79	+1 88
	Cutover	77	18	5	6 0	4 91	
21	Grasses 5 yr., some manure	51	35	14	5 6	5 31	+2 55
	Cutover	54	32	14	6 1	2 76	
24	Currants 5 yr., no manure	83	12	5	6 4	4 88	+2 71
	Cutover	86	9	5	5 9	2 17	
43	Grasses 7 yr., with manure	77	14	9	6 0	7 36	+1 86
	Cutover	76	15	9	5 9	5.50	
64	Old farm, idle, manured	56	32	12	5 8	6 24	+2 33
	Cutover	65	23	12	5 8	3 91	
65	Cereals 2 yr., no manure	68	26	6	6 1	5 12	−0 83
	Stump pasture	59	29	12	6 1	5 95	
Average for the series { c		68	23	9	6 0	5 98	+2 08
v		70	21	9	6 0	3 90	
	Kitsap series						
46	Rotation 25 yr , with potatoes	51	42	7	6 0	4 86	−1 86
	Cutover	64	29	7	6 4	6 72	
49	Rotation 30 yr	25	42	33	5 7	5 10	+3 19
	Cutover	20	37	43	5 4	1 91	
52	Rotation 18 yr , with manure	23	51	26	5 9	5 21	+0 21
	Cutover	24	54	22	5 9	5 00	
53	Rotation 15 yr , with manure	16	45	39	5 8	6 41	+1 72
	Cutover	20	52	28	5 7	4 69	
Average for the series { c.		29	45	26	5 9	5 40	+0 82
v.		32	43	25	5 9	4 58	
	Lynden series						
1	Garden 10 yr., little fert.	77	18	5	6 0	3 79	−0 43
	Stump pasture	77	17	6	5 8	4 22	
47	Cereals 8 yr., no manure	72	23	5	6 0	7 69	+0 93
	Cutover	61	35	4	5 7	6 76	
54	Cereals 3 yr., no fert.	71	22	7	5 9	2 45	+0 90
	Cutover	72	18	10	6 0	1 55	
55	Rotation 30 yr., no fert.	69	25	6	6 1	2 65	+0 60
	Cutover	74	20	6	6 0	2 05	

TABLE 3—*Concluded*

SAMPLE NUMBER	AGRICULTURAL HISTORY	SAND	SILT	CLAY	pH VALUE	ORGANIC MATTER Amount	Change
	Lynden series—*Concluded*						
		per cent	per cent	per cent		per cent	
70	Garden 3 yr., no fert.	72	20	8	6.0	3.52	−0.81
	Cutover	69	22	9	5.9	4.33	
72	Rotation 30 yr., no fert.	68	24	8	6.1	4.84	+2 65
	Cutover	68	26	6	6.0	2.19	
94	Hay 20 yr., with manure	81	15	4	5.8	4.33	+1.19
	Cutover	72	24	4	6.0	3.14	
95	Strawberries 13 yr., some manure	80	16	4	5.7	4.50	+1.95
	Cutover	81	15	4	5.9	2.55	
96	Raspberries 3 yr., no fert.	81	14	5	6.4	1.79	+0.63
	Cutover	78	17	5	6.4	1.16	
112	Hay 25 yr., some manure	60	31	9	5.4	8.84	+5.84
	Cutover	49	38	13	5.4	3.00	
119	Alfalfa 5 yr., no fert.	49	36	15	6.1	8.52	+2 06
	Second growth	44	41	15	5 6	6.46	
121	Rotation 40 yr., with manure	39	46	15	5.7	10.52	+3 44
	Cutover	39	46	15	5 6	7.08	
123	Orchard 24 yr., with manure	63	28	9	5.6	6.90	+1.54
	Stump pasture	57	33	10	5.6	5 36	
Average of the series {c...................		68	24	8	5.9	5.41	+1.58
{v...................		65	27	8	5.8	3.83	
	Miscellaneous series*						
25	Clover 2 yr., no manure	79	18	3	6 0	4.88	−1.20
	Cutover	78	19	3	5 7	6.08	
75	Rotation 17 yr., no manure	78	16	6	5.9	5.03	+1.69
	Cutover	79	16	5	6.3	3 34	
2	Rotation 10 yr., with manure	75	18	7	5 5	6.24	+1.41
	Cutover	83	10	7	5 6	4.83	
97	Strawberries 14 yr., heavy manuring	70	23	7	5.7	3.53	+1.63
	Cutover	75	19	6	5.9	1.90	
16	Rotation 10 yr., no manure	44	44	12	6.2	7.21	+0.92
	Stump pasture	36	48	16	5.8	6.29	
17	Rotation 5 yr., no manure	43	35	22	6 0	5.00	−1.10
	Second growth	42	36	22	5.9	6.10	
Average of the series {c...................		65	26	9	5.9	5.32	+0.56
{v...................		66	25	9	5.9	4 76	

* Series names not yet approved by Bureau of Chemistry and Soils.

three cases. Ordinarily, cutover areas are covered with a ground shrub vegetation with scattering small trees. In this table second growth refers to a well established stand of trees that practically monopolizes the land sur-

face. Stump pastures are usually partly covered with a grass vegetation and are not in as truly virgin condition as other uncropped areas.

Upon averaging the cropped and virgin samples of the Alderwood series, remarkable uniformity in texture and pH values for cropped and virgin soils is indicated. The average increase in organic matter as a result of cropping, however, is 1.27 per cent, which is approximately a 25 per cent increase over the original amount present. This appears to be a significant change. Examination of individual samples which have been cropped for more than 40 years indicates that the improvement through cropping is relatively permanent, especially when good soil management is practiced. No depletion in this period of cropping is suggested.

Samples of the closely associated Everett series of soils, though fewer in number because of less widespread distribution, are even more improved as a result of agricultural use. Virgin samples of this soil average lower in organic matter than virgin Alderwood samples, and cropped samples average slightly higher. With larger numbers this difference might become smaller, but the trend is decidedly toward improvement in organic matter supply as a result of cropping. Only one pair of soils showed more organic matter in the un-cropped sample, and this result may have been influenced by the fact that a stump pasture was selected to represent virgin conditions.

Four sets of samples of the Kitsap series, which are characteristically finer textured than the preceding series, indicated a less marked tendency toward improvement under cultivation, although the soil cropped for 30 years had built up its organic constituent considerably.

The Lynden series represents a group of level soils developed mainly on terraces and old outwash plains. The textures are predominantly sandy, although occasionally finer textured members are found. Thirteen pairs of samples, when averaged, indicated a strong improvement in the supply of organic matter under cropping, but two individual samples showed depletion. Both of these soils were used for home gardens with little or no fertilizer. In general the older cropped soils that had received manure had improved most. The original supply of organic matter in cutover land is exceptionally low in these soils.

A group of miscellaneous soils representing three series, each of limited distribution and at present bearing names which have not been approved, has been included. With two exceptions—soils but a short time under culti-vation—the usual increase in organic matter as a result of cropping was found.

If the entire group of 73 cropped soils is averaged, the organic matter is equal to 5.72 per cent, and the corresponding average for virgin soils is 4.45 per cent. The difference in favor of the cropped soils, 1.27 per cent, is roughly a 28 per cent increase over the amount present in the beginning. Although there are a number of individual samples not in agreement with this general conclusion, the trend toward improvement in the amount of organic matter with cropping in the upland soils of western Washington is unmistakable.

This is not in general agreement with many of the results reported in the humid forested region in the eastern part of the United States. The different character of the climatic environment may have something to do with this difference.

Quality of organic matter

Data have been obtained for total nitrogen and on rate of nitrification on only seven pairs of samples. These results, however, are probably indicative of the quality of the organic matter in cropped and virgin soils of the region. Results of these analyses, together with results of carbon determinations, are given in table 4.

TABLE 4

Nitrogen-carbon ratios and rate of nitrification of certain cropped and uncropped upland soils in western Washington

SAMPLE		N	C	N:C ratio	AMOUNT NITRATE AFTER INCUBATION		
No.	Years cropped				0 days	10 days	30 days
		per cent	per cent		p.p.m.	p.p.m.	p.p.m.
2	10	0 27	3 62	13 40	20	30	80
	0	0 13	2.80	21 54	2	10	80
6	30	0 25	4 34	17 36	3	30	55
	0	0.12	2 89	24 10	0 5	6	20
20	5	0 26	5 03	19 34	1	33	55
	0	0 17	3 65	21.47	0	trace	8
21	3	0 13	3 08	23.70	trace	5	40
	0	0 08	1.60	20 00	trace	2	15
25	2	0.11	2 83	25 73	2	20	60
	0	0 17	3 53	20 76	1	12	120
40	20	0.39	4 94	12 66	2	60	120
	0	0 18	3 31	18 40	1	5	72
42	5	0.12	2.66	22.16	1	20	50
	0	0 10	2 64	26 40	1	10	20

Total nitrogen and carbon vary in amount from soil to soil, and the ratio of these constituents to one another is not a constant. In five soils cropping has resulted in a narrower ratio, but in two soils the reverse was found. In most cases nitrification is more active during a 30-day incubation period in those soils with narrower N:C ratios. This tendency to build soil organic matter with a narrow ratio is probably the result of the gradual replacement of the woody fibrous forest organic matter with the more easily broken down cereal type derived from ordinary crop residues and from manures. This type of organic matter accumulates more readily within the upper soil horizon and is more useful to agricultural plants upon decomposition. During an agricultural history of at least 45 years, the organic matter in the upland soils of western Washington has been increasing under average soil manage-

ment practices. In this respect they have had an unusual agricultural history as compared to soils in eastern Washington and in other parts of the United States.

SUMMARY

Studies of cropped and virgin soils, with especial attention to the changes that have taken place in the organic portion, have been made on 73 pairs of samples representing seven soil series. The agricultural history of each cropped soil was obtained.

The results indicate that, contrary to the previously reported data, there has been an increase in the quantity of organic matter under cropping in western Washington. For all samples examined this increase amounts to about 28 per cent.

The quality of the organic matter is much improved as a result of cropping. This is probably associated with the change in the soil from the forest type to the cereal type of organic residue.

The greatest increases in quantity and quality of organic matter as a result of cropping occur under the better systems of soil management, which add organic material to the soil regularly. Under such management, western Washington soils have maintained a superiority over virgin soils for at least a 45-year cropping period.

REFERENCES

(1) ALWAY, F. J. 1909 Changes in the composition of the loess soils of Nebraska caused by cultivation. Nebr. Agr. Exp. Sta. Bul. 111.

(2) BAKER, G. O. 1936 A study of the practicability of the Walkley and Black method for determining soil organic matter. *Soil Sci.* 41: 47–51.

(3) BOUYOUCOS, G. J. 1929 A new simple and rapid method for determining the moisture equivalent of soils and the role of soil colloids on the moisture equivalent. *Soil Sci.* 27: 233–241.

(4) BOUYOUCOS, G. J. 1930 A comparison of the hydrometer method and the pipette method for making mechanical analysis of soils with new directions. *Jour. Amer. Soc. Agron.* 23: 747–751.

(5) NIKIFOROFF, C. C. 1937 The inversion of the great soil zones in western Washington. *Geogr. Rev.* 27: 200–213.

(6) SIEVERS, F. J., AND HOLTZ, H. F. 1922 The silt loam soils of eastern Washington and their management. Wash. Agr. Exp. Sta. Bul. 166.

(7) SNYDER, H. 1898 Effects of the rotation of crops upon the humus content and the fertility of soils. Minn. Agr. Exp. Sta. Bul. 53.

(8) WALKLEY, A., AND BLACK, T. A. 1934 An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci.* 37: 29–38.

(9) WHEETING, L. C. 1936 Shot soils of western Washington. *Soil Sci.* 41: 35–45.

THE LAWS OF SOIL COLLOIDAL BEHAVIOR: XX. THE NEUTRAL SALT EFFECT AND THE AMPHOTERIC POINTS OF SOILS

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INTRODUCTION

In two previous papers (4, 5), the power of various neutral salts to increase the capacity of a soil to bind acids and bases was investigated. By titrating the soil in water and in a salt solution, two titration curves were obtained which intersect each other at a pH which depends upon the amphoteric nature of the soil and upon the nature of the ions of the salt. The point of intersection represents that pH of the soil suspension which is not affected by the addition of the salt. It is, in other words, the point of exchange neutrality. In cases where the point of intersection corresponds to the point of complete net unsaturation of the soil, it would serve as a point of origin for the determination of the degree of saturation and the combining capacity. For the soil then studied (a podzol B_2 sample), the use of an alkali sulfate seemed to result in the closest agreement.

THE PODZOL

Our next object was to study the results obtained with different soil materials. For this purpose, we first selected samples from the different horizons of the entire podzol profile, representing a wide range with respect to the amphoteric points. The samples were from the previously described Häggbygget podzol profile (3), and the titration was made with H_2SO_4 and KOH in water and in 0.5 N K_2SO_4. The following is a report of this work.

The A_0 horizon is divided into F_1 (slightly transformed förna or litter), F_2 (considerably transformed), and H (structureless humus). In addition to these, the ground vegetation (chiefly Vaccinium) was included and is designated by the symbol F_0. All organic matter was ground and passed through a 2-mm. sieve. The mineral samples, which originally consisted of a fine sandy material, were sifted through a 1-mm. sieve. The coarse and compact material of the stony moraine at 70 cm. was not included because of its different origin. The profile has, strictly speaking, no C horizon.

Figures 63 to 70 show the curves obtained by the electrometric titration of
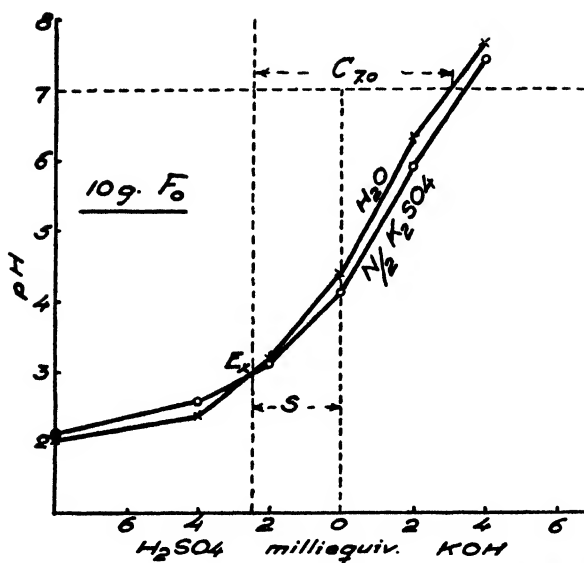
FIG. 63. THE TITRATION IN WATER AND IN 0.5 N K_2SO_4 OF SAMPLES FROM THE F_0 LAYER OF A PODZOL PROFILE
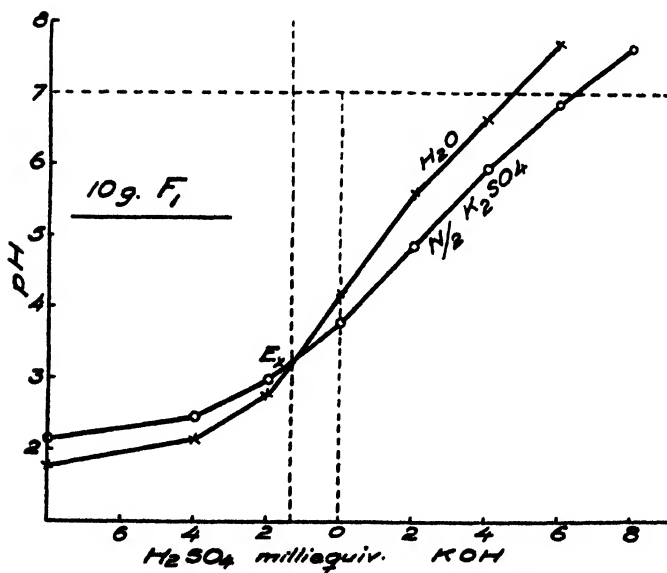
FIG. 64. THE TITRATION IN WATER AND IN 0.5 N K_2SO_4 OF SAMPLES FROM THE F_1 LAYER OF A PODZOL PROFILE
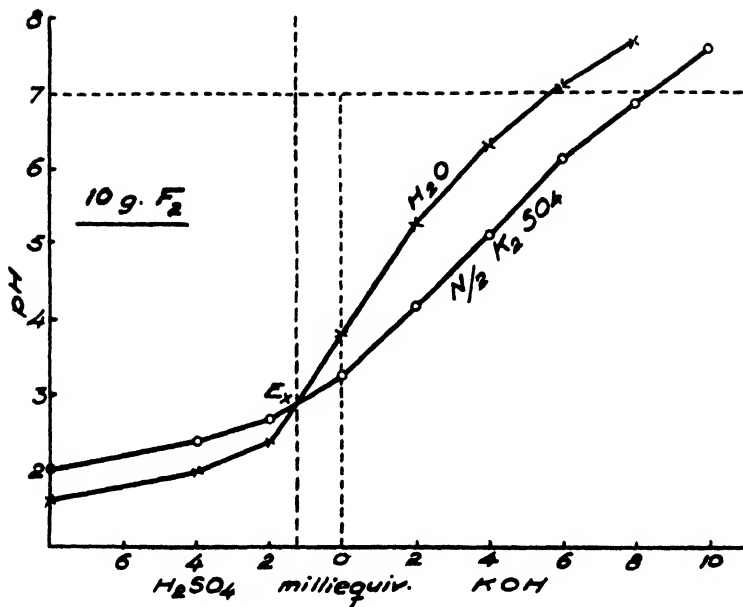
FIG. 65. THE TITRATION IN WATER AND IN 0.5 N K$_2$SO$_4$ OF SAMPLES FROM THE F$_2$ LAYER OF A PODZOL PROFILE
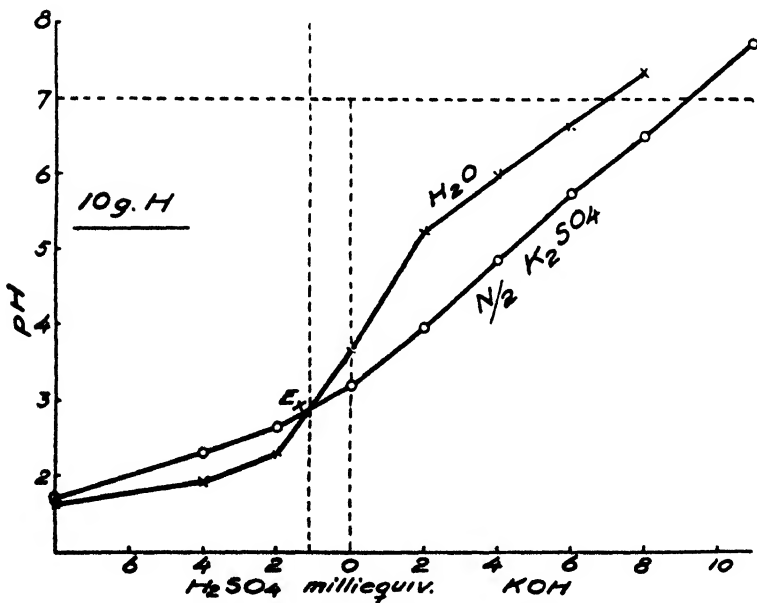
FIG. 66. THE TITRATION IN WATER AND IN 0.5 N K$_2$SO$_4$ OF SAMPLES FROM THE H LAYER OF A PODZOL PROFILE
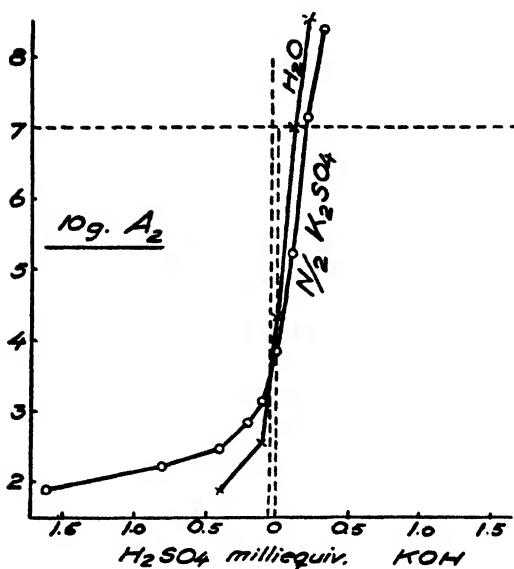
FIG. 67. THE TITRATION IN WATER AND IN 0.5 N K$_2$SO$_4$ OF SAMPLES FROM THE A$_2$
HORIZON OF A PODZOL PROFILE
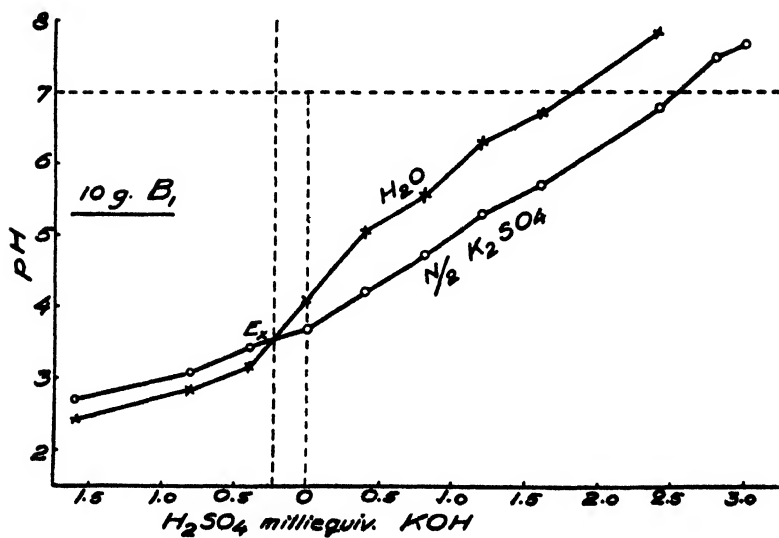
FIG. 68. THE TITRATION IN WATER AND IN 0.5 N K$_2$SO$_4$ OF SAMPLES FROM THE B$_1$
HORIZON OF A PODZOL PROFILE
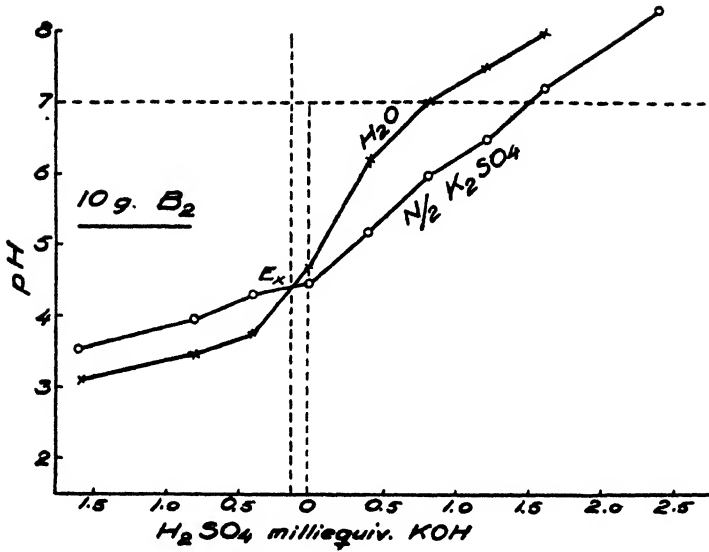
FIG. 69. THE TITRATION IN WATER AND IN 0.5 N K_2SO_4 OF SAMPLES FROM THE B_2
HORIZON OF A PODZOL PROFILE
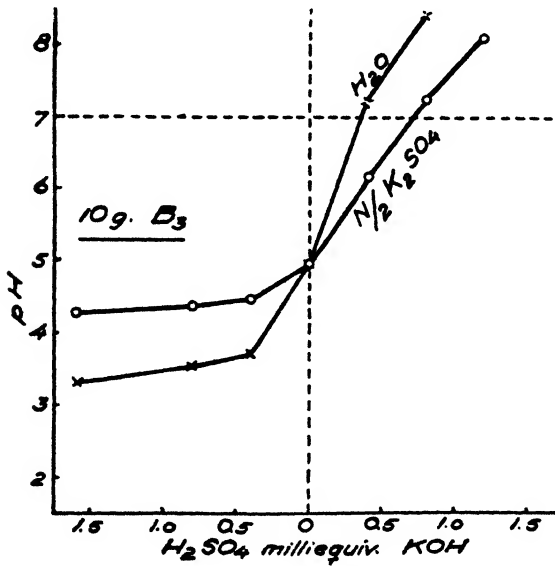
FIG. 70. THE TITRATION IN WATER AND IN 0.5 N K_2SO_4 OF SAMPLES FROM THE B_3
HORIZON OF A PODZOL PROFILE

the different samples in water and in 0.5 N K_2SO_4 solution. Samples of 10 gm. were shaken over night in rubber stoppered test tubes with 50 cc. of a solution containing the various quantities of acid and base. The pH was determined by the quinhydrone method.

There can be no doubt that the intersections of the curves are related to the amphoteric nature of the soil complex, but the true relationship between the point of intersection (i.e. the pH of exchange neutrality) and that pH at which the capacities of the soil to combine with acids and bases are equal is very complicated and will be left for discussion at the end of this paper. This latter pH was previously defined (4) as the equi-ionic point, but then it was identified with the pH of exchange neutrality, with which it coincides only under a special set of conditions, to be explained later. It is, therefore, better

TABLE 151

The E_x, pH_u, S, and C_7 values (see fig. 63), the electrodialyzable bases and acids, the loss on ignition, and the acid-oxalate soluble silica and sesquioxide in the Häggbygget podzol profile

HORIZON	F_0	F_1	F_2	H	A_2	B_1	B_2	B_3
DEPTH OF SAMPLE (CM.)	—	<1	1-4	6-10	13-22	25-28	35-40	50-60
E_x	2.99	3.20	2.89	2.85	3.50	3 53	4.42	4.94
pH_u	2.90	2.88	2 86	2 86	3 86	3 92	4 51	4.78
S m.e./100 gm.	25.0	13.5	12 5	11.2	0 5	2.3	1.2	0 0
C_7 m.e./100 gm.	55 0	60 5	71 0	81 5	1 5	20 5	9.0	3 5
Electrodialysis:								
Bases m.e./100 gm	30 80	20.60	12.45	8 40	0.22	0.37	0.30	0 20
Acids m.e./100 gm	17 90	8.00	3.50	1.86	0.15	0 40	0 26	0 18
Base/acid	1 72	2 58	3 56	4.52				
Loss on ignition (%)					1.03	9 94	7.12	3 90
Acid-oxalate extract:								
SiO_2 (%)					0.17	0 95	0 75	0.75
Al_2O_3 (%)					0 65	1 89	2 13	1.93
Fe_2O_3 (%)					0 26	3 44	3 76	1 64

to retain both terms and keep them distinct. We shall, for the present, designate the point of intersection of the titration curves by the symbol E_x.

If E_x were the true equi-ionic point, then the net saturation S of the soil with bases would be represented by the distance (expressed in m.e. H_2SO_4) between the vertical lines through E_x and O in the figures. Similarly, the capacity of the soil to bind base at pH 7.0 (C_7) would be represented by the distance on the pH 7.0 ordinate between the vertical line through E_x and the "water" titration curve (cf. fig. 63). These distances have been read from the original graphs and are shown as S and C_7 values, together with the E_x values in table 151. For comparison with these values, the pH_u (the pH of the electrodialyzed soil) and the electrodialyzeable acids and bases are also given. The last two series of values were obtained about a year earlier from a different subsample but from the same batch of soil. The table shows also the loss on

ignition and the acid-oxalate soluble silica and sesquioxides (Tamm) of the samples from the mineral horizons. The acid-oxalate extraction deviates from the one recommended by Tamm (7), in that the flasks were placed on the steam bath for about an hour when it was found that the B_1 and B_2 material did not bleach in the cold. All samples received, however, the same treatment.

The E_x values are higher than pH_u in all the F samples, whereas the reverse is true for the mineral samples, with the exception of B_3. On the basis of previous work (4) as well as on theoretical grounds, to be explained later, we should have expected the E_x values to be lower than the pH_u, and that this difference would be greatest in the case of the more acidic organic colloids. In the case of the highly unsaturated mineral samples, the difference $pH_u - E_x$ is greatest in the most acidic A_2 and B_1 samples, is smaller in B_2, and has the opposite sign in B_3, where E_x is higher than pH_u. This is all as one might have expected. The deviation from this "rule" in the case of the organic samples might be due to their relatively high degree of saturation, chiefly with Ca ions. We shall discuss this influence later in connection with our experiment with a rendzina soil.

In comparing the S values with the dialyzeable bases, we find only a very general agreement, as might be expected. From the data at hand, it is impossible to estimate how much of the dialyzeable base existed in combination with the colloidal complex and how much with the soluble acids. The dialyzeable acids are to a large extent weak acids, a certain percentage of which do not function as acids at pH 3 or 4, although they do so at pH 7. This fact should, perhaps, be emphasized.[1] The bases in combination with the soluble acids of the soil do not belong to the exchangeable bases of the colloidal complex and should be subtracted. But even the S values include some of these bases in so far as the acids in combination are weak enough to be displaced by H_2SO_4 above the point of intersection. The S values will also be too large or too small in proportion to the extent that the E_x values deviate to the acid or alkaline side of the true equi-ionic point, respectively. The calculation, on the basis of the S values, of the percentage saturation with reference to the capacity at pH 7 (C_7) yields 33.3, 11.2, and 13.3 for the A_2, B, and B_2 samples, respectively. In view of the corresponding small amounts of dialyzeable bases, these percentages are apparently too high. It should be pointed out, however, that small amounts of electrodialyzeable bases can be determined by titration only very inaccurately, because the presence of an amphoteric complex in the cathode solution makes the endpoint for the strong

[1] After the meeting of one of the sections of the Third International Congress of Soil Science, one of the authors was approached by a young chemist who "did not wish to embarrass the speaker at the meeting" but who felt he ought to call his attention to a "discrepancy" in a table, which showed that the pH of spruce needles was lowered by electrodialysis from 4.02 to 3.74 although more acid (76.6 m.e.) than base (71.3 m.e.) had been removed (1).

bases very uncertain. The average ultimate pH of eight other podzol B samples was, however, found to be higher than the average actual pH. This indicates a negative net base saturation (2).

The loss on ignition and the composition of the acid-oxalate soluble gels show a progressive decrease in the acidoid/basoid ratio from B_1 to B_3. This is reflected in the increase in the E_x and pH_u values in the same order. The acidoid/basoid ratio in A_2 appears lower than in B_1, but it is safe to assume that the acidic surfaces of the bleached mineral particles in A_2 contribute a large part to the acidic activity in the soil.

If we compare the different curves, we note that the salt effect is relatively small in the slightly transformed F_1 material and is still smaller in F_0. This reduced salt effect is undoubtedly caused by the presence of the dialyzeable acids. Some of these acids exist in the uncombined condition and add, therefore, to the titration values. But the neutral salt produces no special effect, beyond the activity effect, on the titration of these acids, which form soluble salts. If the electrodialyzed F_0 material were titrated, a smaller amount of base would be required and the salt effect, remaining approximately the same, would become relatively larger.

The capacity of the colloidal acidoid to bind base is increased by the process of humification, the increase being even greater than indicated by the C_7 values, which include more soluble acids in the younger, than in the older, samples. In a previous work (1), this capacity of the electrodialyzed samples was found to increase from 26 to 205 m.e./100 gm. in spruce needles and from 52 to 173 m.e. in beech leaves, when the green material was compared with the black humus.

THE CAPACITY TO BIND BASE

In figure 71 (a), we have plotted the ratios of the capacity of the soil to bind base in 0.5 N K_2SO_4 to the capacity in water against the number of pH units above the E_x point. We have omitted F_0 and F_1 because of the aforementioned deviation, and we have omitted A_2 because, due to the small capacities, the errors would here be too large. We note that the curves are close together, the ratios being approximately the same at the same distance above E_x. The salt effect is relatively greatest near E_x, where the ratios C_{salt}/C_{H_2O} are greater than 2. At 3 pH units above E_x, the ratios are about 1.5 and approach unity at higher pH. This is all according to theory, because the salt does not increase the absolute capacity of the acidoid to bind base; it only causes the combination to take place at a lower pH. When the acidoid is fully saturated, the salt produces no effect. Due to the compound nature of soils, this point will probably be indefinite and without quantitative significance. A comparative study might, nevertheless, give us a deeper insight into the chemistry of soils.

When we compare the capacity of the soil to bind base, in water and in a salt solution, we can easily account for the large quantities of alkali liberated when a solonchak is changed to a solonetz by leaching.

THE EXCHANGE ACIDITY

In figure 71 (*b*), we have plotted the exchange acidity (the vertical distance between the water curve and salt curve on the right side of E_x) against the number of pH units above the E_x point. We find that the exchange acidity attains a maximum at about 2.5 to 3 pH units above this point. Since the exchange acidity is zero at E_x, due to an equal adsorption of anions and cations, and since it must again be zero when the acidoid is saturated, the observed maximum is a necessary consequence. The maximum exchange acidity is determined by the dissociation constant of the saloid which the acidoid forms with the cations of the salt and by the concentration of the latter. The lower this constant and the higher the concentration of the salt, the greater
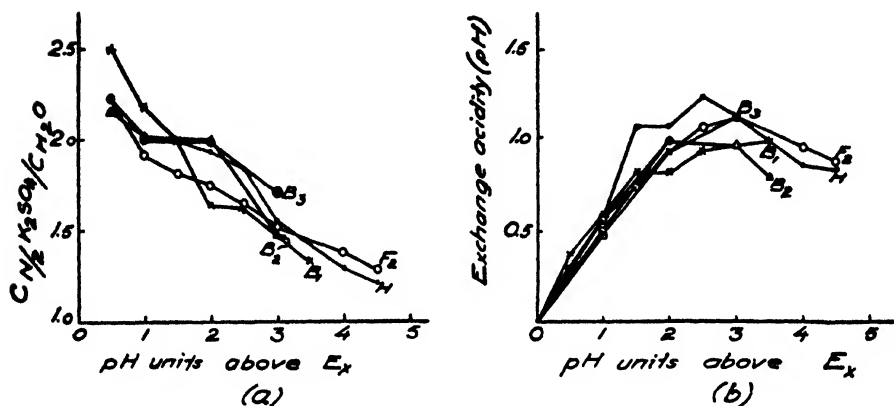
FIG. 71 (*a*). THE RATIOS OF THE CAPACITY TO BIND BASE IN 0.5 N K$_2$SO$_4$ TO THE CAPACITY TO BIND BASE IN WATER AT VARIOUS pH UNITS ABOVE THE POINT OF EXCHANGE NEUTRALITY OF SAMPLES F$_2$, H, B$_1$, B$_2$, AND B$_3$. (*b*) THE EXCHANGE ACIDITY OF THE SAME SAMPLES IN 0.5 N K$_2$SO$_4$ AT VARIOUS pH UNITS ABOVE THE POINT OF EXCHANGE NEUTRALITY

will be the maximum in exchange acidity. For 0.5 N K$_2$SO$_4$, we find a maximum of about 1 pH unit—for the, originally, highly unsaturated soil samples here used.

THE RENDZINA

It is said that there is no rule without exceptions. The soil student is accustomed to finding mostly exceptions. As long as we find everything according to the rule, we have nothing new, once the rule is established. But the exception, if explained, leads to new truths, allowing a deeper penetration into the nature of things. The rule gives us the material for a thesis, the exception creates an antithesis, and on the basis of both of these we construct our synthesis. In the following experiment, we met with nothing but exceptions, but the results are perhaps no less important.

We wanted to study the neutral salt effect on a soil which is the opposite to the podzol, namely, the rendzina. The sample used was from the A horizon in an uncultivated slope, to the west of Båstad, on the west coast of southern Sweden. The soil consists of a black layer sharply changing, through brown, into a white marl at 60 cm. The sample lost 29.4 per cent on ignition and contained about 40 m.e. exchangeable Ca per 100 gm. The pH was 6.98.

On adding 0.5 N K_2SO_4 to this soil, there was a slight exchange acidity (pH 6.83), but upon titration with H_2SO_4, the salt solution soon gave rise to an exchange alkalinity. The curves intersected at a pH of 6.57, at which only 0.25 m.e. H_2SO_4 had been added to 10 gm. of soil. There was obviously something wrong. We accounted for this anomalous behavior as follows.

In the presence of the salt, the high SO_4-ion concentration causes a precipitation of $CaSO_4$ and withdraws the Ca ions from competition with the K ions. The soil becomes, therefore, largely K-saturated and, since the K complex is more hydrolyzed than the Ca complex, this caused the pH to be higher in the sulfate solution than in water, in which the Ca ions were withdrawn from the soil only to the extent to which H_2SO_4 was added. A sulfate, apparently, cannot be used when the soil is Ca-saturated.

Titration with HNO_3, in the presence of 0.5 N KNO_3, yielded at first a moderate exchange acidity, but below pH 5.5, the two curves ran very close to each other, showing no definite point of intersection. The reason for the slight difference in path, at low pH, of the "water" curve and the salt solution curve is that the "water" curve itself becomes, in cases like this, when a saturated soil is titrated with an acid, a salt solution curve. In such cases, the "water" curve will dip down toward the salt solution curve, the dip being greatest when the soil has a high base exchange capacity and is saturated with divalent cations, whereas the salt solution is that of a monovalent cation. An understanding of this behavior is essential when we study titration curves of soils. If, for example, we titrate an unsaturated soil with a base and then titrate it backwards with an acid, we shall find a hysteresis, which depends upon the greater capacity of a soil to bind base in a salt solution and rests, therefore, on a purely chemical basis. When we titrate backwards with an acid, we do so in a continually increasing concentration of salt. The curve will, therefore, sag below the curve obtained by direct titration with a base.

In order to reduce this effect, we decided to try a more concentrated salt solution with a divalent cation, such as 1.0 N $Ca(NO_3)_2$. The result was a decided exchange acidity over the entire range of titration. There was no intersection of the curves down to a pH of 2, at which 7 m.e. acid had been added to 10 gm. of soil.

For soils whose complex is as acidic as that of the rendzina used in our present experiments, it appeared necessary to use a divalent anion in order to get an intersection of the "water" and salt solution curves. It will be recalled that the exchange alkalinity registered by the much more basic podzol B material was not so pronounced when salts of monovalent anions were used (4).

We decided, therefore, to return to our experiment with the SO_4 ions.   To do this, the Ca ions had first to be displaced from the soil, preferably by an alkali cation.   But since the alkali humate was soluble, we had to content ourselves with the Mg-saturated soil, prepared by leaching with $MgCl_2$ and washing. The soil was then titrated by H_2SO_4 in water and in 4.0 N $MgSO_4$.   Evidently because of the high salt concentration, the result was an even greater exchange acidity than in the case of the $Ca(NO_3)_2$, and there was no intersection as far down as pH 1.5, where the curves, however, came very close together.

The basic residue of this soil appears to be too weak to produce an exchange alkalinity, as expressed by the intersection of the two curves.   But if the soil possesses even the weakest basic residue, that is, if it is at all amphoteric, it must have an equi-ionic point.   Why then, do not the curves intersect? We shall attempt to answer this question by an application of the known laws of simple ampholytes, which we believe to be applicable, in a qualitative sense, to the otherwise much more complicated reactions of the ampholytoids.

THE EQUI-IONIC POINT AND THE POINT OF EXCHANGE NEUTRALITY

The definition of *the equi-ionic point* of a soil, in a given solution, is the same as the definition originally given for the pH of exchange neutrality (6).   *It is that pH of a solution which is not affected by the addition of the completely unsaturated soil (free acid-base ampholytoid).*   It represents the pH at which the *capacities* of the soil to combine with the anions and the cations of the solution are equal.

The pH of exchange neutrality, in our experiments the point of intersection of the titration curves in water and in the salt solution, *is that point at which the addition of a neutral salt to a soil suspension does not affect the pH of the latter.* It represents the pH at which the *increments*, produced by the salt, in the capacities of the soil to combine with the anions and the cations of the solution are equal, irrespective of the total capacities at this point.

We shall now, by means of a few theoretical curves, illustrate this rather complicated relationship.

Let us assume an ampholytoid whose apparent acid and base dissociation constants are $K_{a_1} = 10^{-5}$, $k_{a_2} = 10^{-6}$, $k_{a_3} = 10^{-7}$, and $k_{b_1} = 10^{-9}$, $k_{b_2} = 10^{-10}$, $k_{b_3} = 10^{-11}$ when titrated in water by a strong base and acid, respectively, and whose corresponding constants are $k'_{a_1} = 10^{-4}$, $k'_{a_2} = 10^{-5}$, $k'_{a_3} = 10^{-6}$, and $k'_{b_1} = 10^{-8}$, $k'_{b_2} = 10^{-9}$, $k'_{b_3} = 10^{-10}$ when titrated by the same acid and base in a solution of their salt.

If we now, as in a previous case (4), calculate the capacities to bind acid, y_1, y_2, y_3 and y'_1, y'_2, y'_3 in water and in the salt solution, respectively, and the corresponding capacities to bind base x_1, x_2, x_3 and x'_1, x'_2, x'_3 and then plot the $\Sigma y, \Sigma y', \Sigma x,$ and $\Sigma x'$ values against the pH, we get the dotted curves in figure 72.[2]

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[2] $x = \cdot \dfrac{1}{1 + \dfrac{[H^+]}{k_a}}$ and $y = \cdot \dfrac{1}{1 + \dfrac{[OH^-]}{k_b}}$

We note that the effect of the neutral salt is to displace the acid titration curve to higher pH and the base titration curve to lower pH; that is, the power of the ampholytoid to bind acid and base is increased by the salt.

By plotting the $\Sigma x - y$ and $\Sigma x' - y'$ values, we get the full-drawn curves in the figure. These values represent the net capacities to bind acid (left) and base (right). At zero $\Sigma x = \Sigma y$, and this is the equi-ionic point E. We note that this point is at pH 5 in both systems; e.g., in water and in the neutral salt solution. The two titration curves intersect, therefore, at the equi-ionic
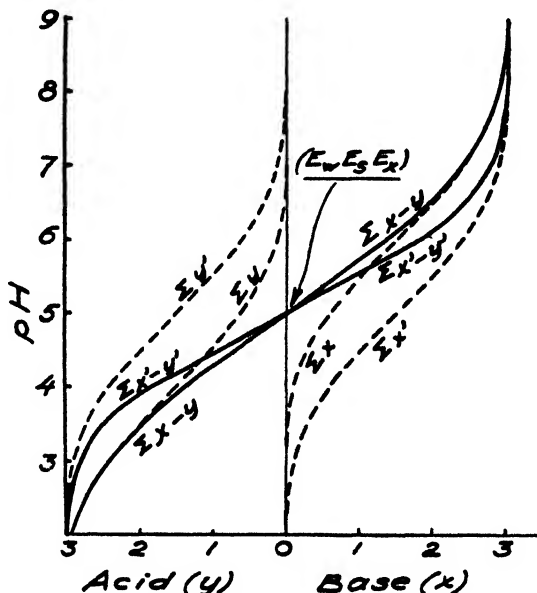
FIG. 72. THE EQUI-IONIC POINT IN WATER E_w AND IN A SALT SOLUTION E_s AND THE POINT OF EXCHANGE NEUTRALITY E_x (ALL AT pH 5) OF AN AMPHOLYTOID WHOSE APPARENT DISSOCIATION CONSTANTS ARE IN WATER:

$k_{a_1} = 10^{-5}, k_{a_2} = 10^{-6}, k_{a_3} = 10^{-7}$, and $k_{b_1} = 10^{-9}, k_{b_2} = 10^{-10}, k_{b_3} = 10^{-11}$

AND IN A SALT SOLUTION:

$k'_{a_1} = 10^{-4}, k'_{a_2} = 10^{-5}, k'_{a_3} = 10^{-6}$ and $k'_{b_1} = 10^{-8}, k'_{b_2} = 10^{-9}, k'_{b_3} = 10^{-10}$

AND WHOSE CAPACITY TO BIND BASE IS x_1, x_2, x_3, AND x'_1, x'_2, x'_3, AND WHOSE CAPACITY TO BIND ACID IS y_1, y_2, y_3, AND y'_1, y'_2, y'_3 IN WATER AND IN THE SOLUTION, RESPECTIVELY

The acidoid and basoid equivalents all $= 1$

point, in this case in which we assumed the salt effect to be the same for the anions and the cations; i.e., E and E_x coincide here.

But suppose that the dissociation constant of the compound (saloid) which the ampholytoid forms with the cations is smaller than the dissociation constant of the compound formed with the anions. The effect of the cations will then be greater, for the same valence, than the effect of the anions; that is, the apparent acid constants and the capacity to bind base will be subjected to a greater increase than the apparent base constants and the capacity to bind

acid.   Figure 73 shows the relationship when the apparent base constants are assumed to be increased only half as much as the acid constants through the action of the salt.   The constants are otherwise assumed to be the same as in figure 72.

It will be noted that the equi-ionic point of the ampholytoid in the salt solution E_s is at about pH 4.75, and not at pH 5.0, the equi-ionic point in water E_w; and that the intersection E_x of the two titration curves is neither at pH 5 nor at pH 4.75, but at a considerably lower pH, or at pH 4.0.   At this pH, the increment in the capacity to bind acid $\Sigma y' - y$ is equal to the increment in the capacity to bind base $\Sigma x' - x$ (or, numerically expressed, $2.0 - 1.5 = 0.6 - 0.1 = 0.5$).
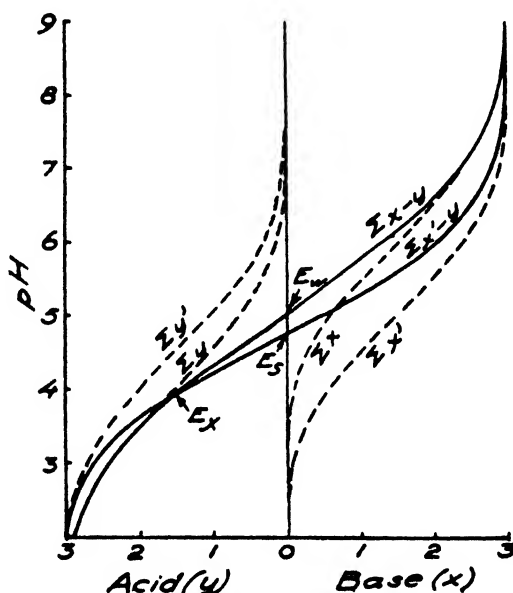
FIG. 73. THE SAME AS FIGURE 72 EXCEPT THAT THE APPARENT BASE CONSTANTS ARE INCREASED BY THE EFFECT OF THE SALT ONLY HALF AS MUCH AS THE APPARENT ACID CONSTANTS

In the above calculations, we have put the concentration of the ampholytoid acid and base $= 1$.   But the colloidal complex can possess any proportion of these groups.

In figure 74, the relationship is shown for the case in which the basic equivalents are one half to one of the acid equivalents.   The constants are all assumed to be the same as in figure 72.   The equi-ionic point in water (E_w) is here at about 4.75 and in the salt solution (E_s) at about 4.45, whereas the titration curves intersect at a pH of about 3.80.

Figure 75 shows the same relationship as is shown in figure 74, when carried a step further.   The constants are all assumed to be the same, but the basic

equivalents of the ampholytoid are put equal to 0.1 to 1.0 of acid. The equi-ionic point in water is here about pH 4.15 and in the salt solution, 3.50. The interesting thing here is that there is no intersection of the titration curves; the curves merely join. The real reason for this is that the pH at which the increments in the capacities to bind acid and base are equal is so low that the basic group is practically saturated without the addition of salt. If we had assumed lower values for k_b and k_a, we would, even here, have gotten an intersection of the curves.

The opposite case, in which the acid equivalence in the ampholytoid is smaller than the basic equivalence, does not exist in soils, because the basoid group (sesquioxide) is itself amphoteric, becoming acidic at high pH. This case need not, therefore, be considered.
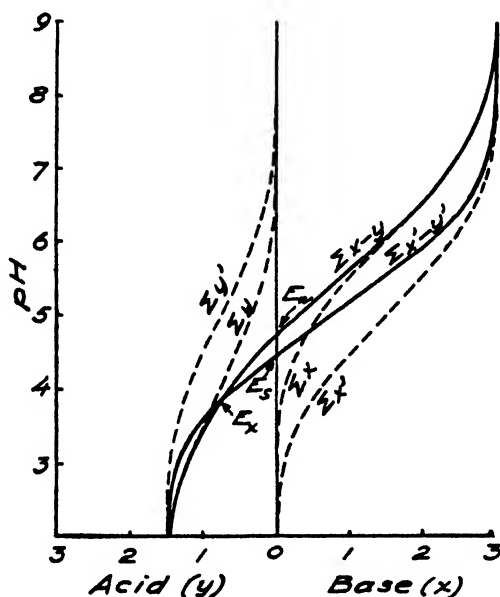
FIG. 74. THE SAME AS FIGURE 72 EXCEPT THAT THERE IS ONLY 0.5 BASOID EQUIVALENT TO 1 OF ACIDOID

The figures bring out the relationship between the different points clearly and allow an exact definition of this relationship in terms of x and y as follows:

$$\Sigma x - y = 0 \text{ (equi-ionic point in water)}$$
$$\Sigma x' - y' = 0 \text{ (equi-ionic point in salt solution)}$$
$$(\Sigma x' - x) - (\Sigma y' - y) = 0 \text{ (point of exchange neutrality)}$$

where x and y represent the capacities to bind base and acid in water, respectively, and x' and y' the corresponding capacities in a salt solution.

The above theoretical analysis has brought out the following relationships:

(A) The equi-ionic point of an ampholytoid in combination with any pair of

ions is the same in water as in a solution of any concentration, provided the effect of the anions and the cations on the capacity of the ampholytoid to bind acid and base is the same, and provided that there is an equivalence between the acidic and basic residues in the ampholytoid, or that these two factors deviate so as to compensate each other.   The titration curves in water and in the salt solution will then intersect at the equi-ionic point, which is then identical with the point of exchange neutrality (cf. fig. 72).

(B) If the effect of the anions and cations differs, or if the equivalence of the acidic and basic residues is different, then an increasing salt concentration will increasingly displace the equi-ionic point to the acid side if the effect of the anions is the weakest (cf. fig. 73) or if the basic equivalence is the smallest (cf. fig. 74 and 75), and to the alkaline side if the conditions are the opposite.
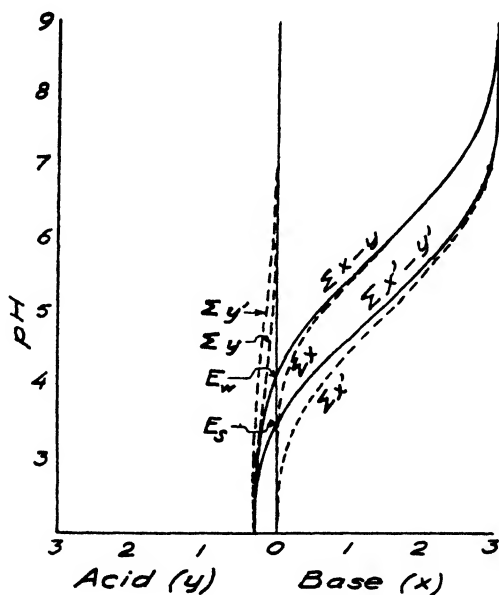
FIG. 75. THE SAME AS FIGURE 72 EXCEPT THAT THERE IS ONLY 0.1 BASOID EQUIVALENT TO 1 OF ACIDOID

The point of intersection of the titration curves, i.e., the point of exchange neutrality, will be on the acid side of the equi-ionic point, in the first case. and on the alkaline side of this point under the opposite conditions.   In extreme cases, the titration curves might not intersect at all but merely join, as in figure 75.

(C) Since the conditions mentioned at the beginning of the preceding paragraph (B) are the most general, it follows that most soils have a different equi-ionic point and a different point of exchange neutrality in solutions of different concentrations.

Soils having a high isoelectric point, such as the red and brown earths, low

in humus, seem to interact with the alkali sulfates approximately according to the relationship shown in figure 72, and with the chlorides more nearly according to figure 73, whereas the interactions of the silicious clays and soils high in humus, which have a low isoelectric point, may be assumed to be more nearly represented by figures 74 and 75.

The intersection of the titration curves, obtained in solutions of different concentrations, apparently promises little as an exact point of origin for quantitative measurements, but we believe that further work in this direction will be fruitful in giving us a deeper insight into the chemistry of soils, as well as into the behavior of colloidal electrolytes in general.

SUMMARY

The study of the neutral salt effect, as expressed by the titration curves of soils, has been continued and applied to podzol and rendzina.

A theory has been advanced regarding the relationship in different soils between the point of intersection of the titration curves obtained in water and in neutral salt solutions (i.e., the point of exchange neutrality) and the point of complete net unsaturation (i.e., the equi-ionic point).

REFERENCES

(1) MATTSON, SANTE, AND EKMAN, PER. 1935 The reaction and the buffer capacity of soil organic matter. *Third Internatl. Cong. Soil Sci.* 1: 374–377.
(2) MATTSON, SANTE, AND GUSTAFSSON, Y. 1934 The chemical characteristics of soil profiles: I. The podzol. *Ann. Agr. Col. Sweden* 1: 33–68.
(3) MATTSON, SANTE, AND GUSTAFSSON, Y. 1935 The chemical characteristics of soil profiles: II. The mutual interactions of podzolic materials. *Ann. Agr. Col. Sweden* 2: 1–30.
(4) MATTSON, SANTE, AND GUSTAFSSON, Y. 1936 The neutral salt reaction in relation to the point of exchange neutrality, the saturation and the combining capacity of a soil. *Ann. Agr. Col. Sweden* 2: 136–157.
(5) MATTSON, SANTE, AND GUSTAFSSON, Y. 1937 The laws of soil colloidal behavior: XVIII. Colloidal electrolytes. *Soil Sci.* 43 (in press).
(6) MATTSON, SANTE, AND HESTER, JACKSON B. 1932 The laws of soil colloidal behavior: X. Exchange neutrality and combining capacity. *Soil Sci.* 34: 459–482.
(7) TAMM, OLOF 1932 Über die Oxalatmethode in der chemischen Bodenanalyse. *Meddel. Statens. Skogsförsöksanst* [Sweden] 27: 1–20.

SOME DATA ON THE MEDITERRANEAN RED SOILS[1]

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DESCRIPTION OF THE SOILS

The samples of soils used in the experiment described here were obtained from Gan-Moshe plantation, situated near the Jewish Colony Rishonle-Zion, Palestine. They are red sandy soils, such as are found on the Mediterranean coast of Palestine, between Zichron Jacob in the north and Gedera in the south. These soils are very well adapted for citrus plantations, because of their excellent physical properties. The chemical and physical properties of their various parts and their characteristic color, varying from reddish gray to dark red, are influenced by their humus content and by the relation between the quantities of sand and clay. The samples represent six profiles and were taken at the arbitrary depths of 0–20 cm., 20–60 cm., and 60–100 cm.

Profile I

The top layer is brown-red, somewhat compact loamy sand and does not give off CO_2 on the addition of acid. The second layer is richer in clay content, more compact in its structure, and likewise contains no CO_2. The third layer appears to be like the second layer.

Profile II

The top layer is of a light brown color and gives off CO_2 on the addition of HCl. The lime is not a constituent of the soil but was added. The second layer is somewhat more compact than the first, brown-red in color, and contains no CO_2. The third layer is compact and contains no CO_2. The color is dark red-brown, with black iron stains indicating a beginning of Nazaz formation.

Profile III

The top layer is the same as that of profile II. The second layer is more compact than the first; the soil is not homogenous, it is red-brown in

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[1] This work is a continuation of a previously published work by F. Menchikowsky and S. Ravikowitch.

[2] The author wishes to express his thanks to the government analyst, G. W. Baker, F.I.C., F.C.S., for his advice and corrections, and to Dr. S. Adler and Dr. A. Rosenberg for their assistance in the analytical work.

color, and contains no CO_2. In the third layer the color is dark red-brown; the soil contains no CO_2 and has many grains of iron oxide, indicating a beginning of Nazaz formation.

Profile IV

The top layer is sandy, contains some organic substance, but does not contain CO_2. The second layer is somewhat more compact than the first, contains no CO_2, has no Nazaz stains, and is red-brown in color.

Profile V

The top and second layers are similar to the corresponding layers of profile IV. The third layer is dark red, contains no Nazaz stains or grains of iron, and does not give off CO_2 on the addition of HCl.

Profile VI

The three layers are similar to those of profile V.

PROCEDURE

The mechanical and physical properties of the soil were investigated. In the chemical investigations the following constituents were determined: the chloride content and the pH in a water extract 1:5, according to Gedroiz (4); in HCl extract, the contents of Ca and K and the relation between these constituents, Ca, Mg, and H in the exchangeable bases; the quantities of iron and aluminum oxides in the clay fraction of the soil.

The following methods were used for the various analyses: the mechanical analysis was made according to the Sudan method worked out by Beam (1); the water-holding capacity was determined according to Keen and Raczkowski (5); the permeability was expressed as liter per hour; the hydrogen-ion concentration was determined according to Clark (2), colorimetric method; and the base exchange, according to Gedroiz (4). The quantity of iron and aluminum in clay was determined in the usual manner, by fusion. Before considering the results of the analyses, we wish to discuss the question of the downward moving clay, which changes the composition of the profiles and exerts an influence on the fertility of the soil.

The grains of the red sandy soil consist of a composition of sea sand and very fine clay particles; both kinds of particles together form a film of red color. Whereas the sand consists of SiO_2, the clay is more complicated, consisting of Fe_2O_3, Al_2O_3, and SiO_2, and also of the exchangeable electrolytes K, Na, H, Ca, and Mg. Apparently, and according to the view of Walter (10) and Ravikowich (8), Ca and Mg form a cementing link between the alluvial clay and sand during the formation of the red sandy soil. The silica and clay are not strongly bound together; it is possible to cause rapid separation between them by the addition of water. The same phenomenon is noticed when a sample of the soil is crushed into a fine powder in an agate mortar. When viewed

through a microscope, the sand and clay particles can be distinguished distinctly.

The property of exchange in the zeolite complex and the lime requirements of the citrus plant should be considered. As our analyses reveal and according to investigations carried out by Oppenheimer (7), the soil is poor in lime, and the plant is therefore compelled to get lime from the zeolite complex. The free hydrogen content in the irrigation water (10^{-7}) which is added to the soil, together with the hydrogen from the carbonic acid in the water, replaces the lime which has been abstracted by the plant from the zeolite complex. Since

TABLE 1

Mechanical composition and moisture content of the various layers

DEPTH		MOISTURE AT 100°C.	CLAY	SILT	FINE SAND	COARSE SAND
	cm.	per cent	per cent	per cent	per cent	per cent
I	0– 20	0 9	14 6	1 0	11 7	72 7
	20– 60	1 8	23 5	1 7	14 5	60 3
	60–100	2 0	24 7	1 8	16 5	57 0
II	0– 20	0 4	6 1	1 0	7 1	85 8
	20– 60	1 6	9.2	0 8	11 4	78 6
	60–100	2 5	24 0	1 0	11 9	63 1
III	0– 20	0 4	9 0	1 1	13 7	76 2
	20– 60	1 0	10 1	1 1	11 5	77 3
	60–100	2 7	22 2	1 0	9 2	67 6
IV	0– 20	0 4	4 9	0 9	11 1	83 1
	20– 60	0 8	8 5	1 3	12 7	77 5
	60–100	1 9	18 3	1 0	11 7	69 0
V	0– 20	0 3	3 9	1 1	14 1	80 9
	20– 60	1 6	16 7	0 9	12 9	69 5
	60–100	1 4	16 8	1 0	13 5	68 7
VI	0– 20	0 5	5 9	1 1	13.3	79 7
	20– 60	1 2	13 4	1 6	13 0	72 0
	60–100	3 2	32 1	4 9	16 4	46 6

this combination (H-clay) is very labile, further addition of water separates the clay from the sand, taking the former to the lower layers and resulting in an accumulation of clay in the lower, and sand in the upper layers.

The accumulation of these materials in the lower portion of the profile gives rise to the compact layer known locally as Nazaz.

DISCUSSION OF RESULTS

The extent of the degradation process can be demonstrated by the following results: the mechanical and physical changes in each layer (table 1); the quan-

tity and composition of the exchangeable cations in the various layers, especially the change in hydrogen; the change in sesquioxide (Fe_2O_3 and Al_2O_3) in the various layers; and the small quantity of lime.

With the increase of clay content with the depth, the physical properties of the soil are also changed (table 2). Table 2 shows that the water-holding capacity of the soil increases with the increase of the clay content; the same

TABLE 2

Physical analysis of samples

DEPTH	TRUE SPECIFIC GRAVITY	APPARENT SPECIFIC GRAVITY	PORE SPACE	WATER-HOLDING CAPACITY	PERMEABILITY
cm.			per cent	per cent	
I { 0- 20	2.60	1.55	40.4	37.9	26h.51*
20- 60	2.55	1.43	43.9	47.0	56h.40*
60-100	2.53	1.63	35.5	47.0	58h.
II { 0- 20	2.63	1.54	41.4	28.4	8h.36*
20- 60	2.61	1.51	42.1	31.8	23h.46*
60-100	2.70	1.38	48.8	47.5	95h.
III { 0- 20	2.72	1.57	42.2	28.5	1h.
20- 60	2.75	1.56	43.6	32.9	1h.
60-100	2.64	1.45	45.0	44 7	125h.
IV { 0- 20	2.71	1.60	40.9	25.9	16h.40*
20- 60	2.75	1.62	41.1	29.4	20h.
60-100	2.67	1.49	43.3	41.0	66h.40*
V { 0- 20	2.84	1.60	43.6	23.8	10h.
20- 60	2.67	1.45	45.2	36.7	33h.20*
60-100	2.75	1.56	43.3	35.9	65h.
VI { 0- 20	2.71	1.58	41.7	27.5	31h.40*
20- 60	2.67	1.48	44.5	34 0	200h.
60-100	2.61	1.47	43.6	48.1	433h.

* 1 liter in hours.

is true in regard to the permeability of the soil. The pore space is expressed by the formula

$$\frac{a - b}{a} \times 100,$$

where a = the true specific gravity,
and b = the apparent specific gravity.
This, of course, does not give a picture of the pore space of the soil *in situ*.

Table 3 shows that in the water extract the chlorine content in all the layers

is very small.   Though the variation in pH is small, there is (except in profile
1) a lowering of the figure in the third layers.   The calcium content in all the
profiles and layers is very small, as was found by Oppenheimer (7).   The soil
is also poor in potassium.   Table 4 shows that the total of the exchangeable
bases increases with the depth.   The same results are expressed in percentages
in table 5.   Table 6 shows the distinct increase of sesquioxide (Fe_2O_3 +
Al_2O_3) in the clay fraction of the various layers.   The figures show that the

TABLE 3

Analysis of water and hydrochloric acid extracts

DEPTH		WATER EXTRACT 1:5		HCl EXTRACT		RATIO OF Ca·K*
		Cl	pH	Ca	K	
	cm.	per cent		per cent	per cent	
I	0– 20	0 002	7.3	0 24	0 099	0 41
	20– 60	0 006	7 3	0 31	0 120	0 39
	60–100	0 006	7 3	0 32	0 110	0 34
II	0– 20	0 003	7.3	0 10	0 160	1 6
	20– 60	0 004	7 3	0 17	0 073	0 45
	60–100	0 008	7 1	0 29	0 160	0 55
III	0– 20	0 002	7 3	0 20	0 099	0.49
	20– 60	0 003	7 3	0 24	0 073	0 30
	60–100	0 005	7 25	0 31	0 096	0 31
IV	0– 20	0 003	7.3	0 17	0.076	0.45
	20– 60	0.003	7 3	0 29	0 085	0.29
	60–100	0 004	7 25	0 32	0 130	0 40
V	0– 20	0.002	7.3	0.13	0 048	0 36
	20– 60	0 003	7 3	0 24	0 120	0 50
	60–100	0 005	7 1	0 28	0 082	0 29
VI	0– 20	0 002	7 4	0 24	0 24	1 00
	20– 60	0 004	7 3	0 24	0 093	0 38
	60–100	0 003	7 1	0 38	0 23	0 61

* Ca is taken as 1.

iron and aluminum content increases with the depth, except in profile I,
where iron and aluminum appear in the second layer in greater quantities
than in the third layer.   The fact that the iron content increases with the
depth conforms with the fact that the iron grains and stains appear in the
lower layers.   This is also due to the difference in solubility and absorption of
aluminum and iron.   More detailed accounts are found in the work of Menchi-
kowski (6) and of Fodor and Rosenberg (3).

TABLE 4
Exchangeable bases expressed in milliequivalents

DEPTH		HYDROGEN	MAGNESIUM	CALCIUM	TOTAL BASE EXCHANGE
	cm.	m.e.	m.e.	m.e.	m.e.
I	0– 20	0 16	3 2	5 99	9.35
	20– 60	2 80	5 8	6.65	15.25
	60–100	3 60	5.3	8 90	17 80
IV	0– 20	0 20	1.2	2.28	3.68
	20– 60	0 32	1.3	3.30	4.65
	60–100	1 64	3.3	6 12	11.06
VI	0– 20	0.40	0 2	0.66	1 26
	20– 60	0 60	1.5	5 70	7 80
	60–100	4 64	6 7	13 02	24 36

TABLE 5
The amount and composition of exchangeable ions

DEPTH	DESCRIPTION AND NUMBER OF PROFILE	PERCENTAGE OF THE VARIOUS BASES IN RELATION TO THEIR TOTAL CONTENT		
		Hydrogen	Magnesium	Calcium
cm.		per cent	per cent	per cent
0– 20	1 A, No. I	1.71	34.22	64 07
20– 60		18.36	38 03	43 61
60–100		20.22	29 77	50.01
0– 20	1 B, No. IV	5.43	32 61	61.96
20– 60		6.88	27.95	65.17
60–100		14 82	29 83	55 35
0– 20	2 A, No. VI	31 74	15.87	52 39
20– 60		7.69	19.23	73.54
60–100		19 05	27.50	53.45

TABLE 6
Sesquioxides in profile

DEPTH		CLAY CONTENT IN THE SOIL	Fe_2O_3	Al_2O_3	$Fe_2O_3 + Al_2O_3$
	cm.	per cent	per cent	per cent	per cent
I	0– 20	14.6	4.15	21.53	25.68
	20– 60	23 5	5.44	28.42	33.86
	60–100	24.7	6.02	23.56	29 58
IV	0– 20	6 1	1.74	3.33	5.07
	20– 60	9.2	2.30	7.76	10.06
	60–100	24 0	5 31	26.32	31.63
VI	0– 20	9 0	1.98	7.75	9.73
	20– 60	10.1	5.09	22.61	27.70
	60–100	22.0	5.79	26.34	32.13

SUMMARY

Eighteen soil samples have been examined in order to investigate their properties in relation to Nazaz formation. The results appear to support the following general statements:

The red-sandsoil called "Hamrah" consists of sand and alluvial clay, which are not firmly held together. The cementing link between them has been produced by the exchangeable bases, particularly the cation Ca.

Since the soil is very poor in lime and since the citrus plants require for their nutrition large quantities of lime, it is abstracted from the zeolite complex, thereby separating the clay from the silica.

Rainfall and irrigation may be expected to have the following effect: The clay penetrates into the lower layers, whereas the sand remains in the upper layers. Since the water contains free hyrogen ions, the hydrogen takes the place of calcium, and consequently, the physical properties of the soil are impaired.

Because of the difference in solubility (and absorption) of aluminum and iron, separation between them sets in, and due to lack of oxygen from the air, the lower layers of the soil bear stains and grains of FeO, the quantity of which increases with the degradation of the soil. This results finally in formation of Nazaz (*Pan*) which may be close enough to the surface to impede cultivation.

The following measures should be adopted to protect the red-sand soil against degradation: The water optimum should first be determined by experiments, thus avoiding surplus of water, which contributes to the separation between the clay and sand. The lime requirements of the plant (when necessary determined by experiment) should be provided for by addition of lime to the soil, thus avoiding the removal of lime from the zeolite complex, which forms a link between the clay and sand.

When applying fertilizers it is of the utmost importance to use organic fertilizers, in order to produce humus which should serve as a protective colloid. As these soils are low in water-soluble salts and lime and are to some extent shaded from direct sunlight in citrus groves, conditions for humus formation are not entirely lacking provided the organic matter is available (9).

REFERENCES

(1) BEAM, W. 1911 The mechanical analysis of arid soils. *Cairo Sci. Jour.* 5: 107-119.

(2) CLARK, M. W. 1926 The Determination of Hydrogen Ions, ed. 2, Baltimore.

(3) FODOR, A., AND ROSENBERG, A. 1928 Über die Absorption von Eisen aus Eisenhydrate solen und aus Eisenchloridloesungen durch Kaolin und Talkum. *Kolloid Ztsch.* 46: 91-95.

(4) GEDROIZ, K. K. 1926 Chemische Bodenanalyse. Gebr. Borntraeger, Berlin.

(5) KEEN, B. A., AND RACZKOWSKI, H. 1921 The relation between the clay content and certain physical properties of a soil. *Jour. Agr. Sci.* 11: 441–449.

(6) MENCHIKOWSKI, F. 1932 Pan (Nazaz) and its origin in the red-sandy soils in Palestine. *Jour. Agr. Sci.* 22.

(7) OPPENHEIMER, H. R. 1934 Problems of citrus nutrition. *Hadar* 7: 268–271.

(8) RAVIKOWICH, S. 1934 The movement of a colloidal clay in red-sandy soil, a factor interfering with normal soil properties. *Hadar* 7: 246–251, 272–275.

(9) REIFENBERG, A. 1929 Die Entstehung der Mediterran Roterde. Th. Steinkopff, Dresden.

(10) WALTHER, J. 1924 Das Gesetz der Wüstenbildung. Von Quelle & Meyer, Leipzig.

COMPARATIVE EFFICIENCY OF CALCINED PHOSPHATES[1]

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One of the most recent and promising processes for rendering phosphorus in phosphate rock soluble or available to plants was developed in the Division of Fertilizer Investigations, Bureau of Chemistry and Soils, U. S. Department of Agriculture (6, 7, 11, 12, 15, 16). Briefly, this new process consists in heating phosphate rock to 1400°C. in the presence of 4 to 10 per cent silica and a reducing atmosphere of water vapor, whereby 95 to 100 per cent of the fluorine naturally found in rock phosphates can be volatilized and 85 to 95 per cent of the total phosphoric acid converted into the citrate-soluble form.

Jacob and co-workers, of the Bureau of Chemistry and Soils (5, 8, 9, 10, 14), have shown that fluorine is an important natural constituent of all grades of phosphate rock mined in the United States and in most foreign countries. The fluorine, which commonly ranges from 3 to 4 per cent (8), may form part of the complex mineral fluorapatite $[Ca_{10}F_2(PO_4)_6]$ and hydroxyfluorapatite $[Ca_{10}(OH,F)PO_4)_6]$, believed by these authors to be the chief minerals in most phosphatic rocks mined throughout the world. Jacob, et al. (6, 7, 8, 9, 11) and Bartholomew (3) have shown respectively that the solubility and the availability to plants of phosphorus in natural phosphates are lower in types that have a high percentage of fluorine than in types that have a low percentage. Whether the low yield of plants is principally due to the insoluble nature of phosphorus in fluorapatite-like substances or to a deleterious effect of fluorine on plant growth is not yet known. Results have been contradictory, but the majority indicate that insolubility of the phosphorus rather than toxicity of fluorine is the chief cause of low yields and of unavailability of phosphorus to plants in most raw phosphates (1, 3, 4, 13). The new calcination process breaks up the fluorine-bearing phosphate compounds into a product the formula of which is not yet known.

The only results with defluorinated calcined phosphates on plants reported to date[3] are those of Jacob, et al. (6, 7). Based on equal applications of phosphoric acid, the results obtained (6) with cabbage at Alabama and with millet

175

(unlimed) at the U. S. Department of Agriculture and at West Virginia indicate that calcined phosphate from Florida land pebble with more than 78 per cent of its original total fluorine volatilized, gave in general better dry-weight yields of crops and higher recovery of phosphoric acid (P_2O_5) by plants than did superphosphate. Likewise, Tennessee brown-rock with more than 97 per cent of its original total fluorine volatilized gave higher yields of oven-dry matter and a greater recovery of phosphoric acid than did superphosphate and, in most cases, dicalcium phosphate. As might be expected, yields of crops and recovery of phosphoric acid were much lower with the untreated Florida pebble and Tennessee brown-rock phosphates than with any of the calcined phosphates.

At the time this work was started only the availability of the calcined phosphates prepared by the Bureau of Chemistry and Soils, as determined by laboratory experiments, was known. During the winter of 1934, pot experiments were conducted at The Pennsylvania State College in order to test the efficiency of two grades of calcined phosphate in comparison with other phosphates.

MATERIALS AND METHODS

Five different phosphate fertilizers, with analyses shown in table 1, were used.

Calcined phosphate A. Calcined phosphate A was prepared from Tennessee brown-rock phosphate as described in several publications of the Bureau of Chemistry and Soils (15, 16). Approximately 2-gm. charges of 40- to 50-mesh Tennessee brown-rock phosphate were heated in a platinum boat in an electric furnace at about 1400°C. As the original rock had the desired content of silica, over 4 per cent, this material was not added to it. During the process of heating, which lasted over half an hour, steam was passed through the rotary kiln at the rate of 1.0 gm. a minute. The sintered or semi-sintered product was cooled rapidly, ground to the desired fineness, and analyzed for total phosphoric acid, for citrate-soluble and water-soluble phosphoric acid. for fluorine, and for other constituents.

Calcined phosphate B. Calcined phosphate B was prepared in much the same way as calcined phosphate A (16). This sample differs from calcined phosphate A in that a smaller percentage of its original total fluorine was volatilized.

Superphosphate. Regular commercial superphosphate was used.

North African phosphate rock. North African phosphate rock was shipped from Morocco to Cornell University, and a sample was sent to be tested in this department. This type of natural phosphate is softer than the domestic raw rock phosphate.

Tennessee brown-rock phosphate. Although not the identical material from which calcined phosphates A and B were prepared, Tennessee brown-rock phosphate was so similar to it that for purposes of comparison this sample was used in the experiments.

As sources of potash and nitrogen Baker's C.P. analyzed potassium chloride (KCl) and sodium nitrate (NaNO₃), respectively, were used.

One-gallon well-glazed crocks were filled with Morrison sandy loam which had been previously passed through a ½-inch screen and carefully mixed. This reddish brown soil was taken at a 2- to 9-inch depth from "The Barrens," near State College. The soil is deficient in nutrient elements, particularly phosphorus. The original pH of the soil was 6.2, as determined by the quin-hydrone electrode.

All phosphate carriers, except superphosphate, were applied on the basis of total phosphoric acid content at the rates of 50 and 100 kgm. (110 and 220 pounds) of phosphoric acid an acre, or 0.2260 and 0.4519 gm., respectively, of total phosphoric acid per crock, each in quadruplicate. Superphosphate was applied on the basis of its citrate-soluble phosphoric acid (16.0 per cent)

TABLE 1

Composition of phosphates used in field and greenhouse experiments

PHOSPHATE MATERIAL	MESH	TOTAL P_2O_5	CITRATE-SOLUBLE P_2O_5		WATER-SOLUBLE P_2O_5	FLUOR-INE	$\dfrac{F_2}{P_2O_5}$
			In sample	In total P_2O_5			
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1. Calcined phosphate A*	80	35 09	32 52	92 6	Trace	0 10	0 28
2. Calcined phosphate B*	80	36 05	23 95	66 4	Trace	0 65	1 80
3. Superphosphate.	80	18 32	16 0†	88 0			.
4. North African rock phosphate*	100	34.22	5 10	14 9	.	4 04	11.80
5. Tennessee brown-rock phosphate*..	100	33 5	2 00†	6 0		3 70	11.04

* Analysis made at the Bureau of Chemistry and Soils, U. S. Department of Agriculture. Samples 1, 2, and 5 were supplied by the Bureau of Chemistry and Soils. Sample 4 was supplied by the department of agronomy at Cornell University, Ithaca, New York.

† Approximate analysis.

instead of its total phosphoric acid. This represented 57 and 114 kgm. of phosphoric acid an acre applied as superphosphate. Nitrogen and potash were each applied at the rate of 50 kgm. an acre, or 0.2260 gm. per crock. All fertilizers were carefully mixed with the entire soil of each crock.

As checks four crocks were fertilized with nitrogen and potash only (N–K), and two were left unfertilized to test the general fertility of the soil.

On November 30, 1935, Patterson oats were seeded at a uniform depth and at uniform intervals in all crocks. Three cropped replicates of each treatment were kept, and one pot of each treatment was left unplanted for soil studies. The moisture content was maintained at 10.5 per cent in all crocks throughout the experiment.

After germination the plants were thinned to 26 in each pot. These were harvested on February 12, 74 days after planting. No plants showed any signs of maturity or flowering.

The green weight of tops and roots combined per pot were recorded. Later,

after the tops were separated from the roots and dried over a steam radiator for 2 days and in an oven at 80 to 90°C. for 8 hours, they were carefully weighed.

Only the tops of plant material were analyzed for total phosphoric acid. It was realized that the roots may contain precipitated phosphate in large quantities (3) and are not therefore such good indicators of the phosphorus used by the plant as are the tops. The material was ignited in a muffle furnace, and phosphoric acid was determined in the ash (2, "Absence of Sulphates").

At the conclusion of the experiment the available phosphoric acid in cropped and uncropped soils was determined by extraction with 0.25 N sodium acetate at pH 5, the color being developed according to the molybdate-tin reaction of Denigès.

RESULTS AND DISCUSSION

Yields. After growing 74 days the crops were photographed and the yields harvested. Measurements were made of the length of tops and roots, green

TABLE 2

Dry weight of oats grown in pots on Morrison sandy loam which received different phosphates at the rate of 110 and 200 pounds P_2O_5 an acre

FERTILIZERS	WEIGHT OF TOPS		RELATIVE EFFICIENCY	
	110 lbs. P_2O_5	220 lbs. P_2O_5	110 lbs. P_2O_5	220 lbs. P_2O_5
	gm.	*gm.*	*per cent*	*per cent*
Calcined phosphate A.....	5 02	5.54	99.0	102 4
Calcined phosphate B.....	4.64	5 18	91.3	95 6
Superphosphate....	5 07	5.41	100 0	100 0
North African rock phosphate.....	4 15	4 23	81.9	78 2
Tennessee brown-rock phosphate..	3.32	3.45	65.5	63.8
N and K only.........................	2 94		57.9	
No treatment.....................	1 06		20 9	

weight of tops, dry weight of tops, and total phosphoric acid content of tops. All of these measurements placed the efficiencies of the different phosphatic fertilizers in the same relative order; therefore only the dry weight and total phosphoric acid recovered are reported (table 2). These data indicate clearly that, insofar as yields are a measure of efficiency, the two calcined phosphates are nearly as available as superphosphate and the untreated rock phosphates are of much lower availability.

Phosphoric acid in plants. The quantity of phosphoric acid assimilated by the plant is, perhaps, a better criterion of availability than is yield. Consequently the plants were analyzed for this constituent. The results are reported as percentage of phosphoric acid in tops and as net phosphoric acid recovered from the phosphates, obtained by subtracting the P_2O_5 content of the N-K pots from that of the pots receiving the various phosphates. Table 3 shows that both as regards percentage composition and net recovery, the

calcined phosphates are practically on a par with superphosphate. Calcined phosphate B is less efficient than A. Here again, as in yields, the rock phosphates are of much lower efficiency than the calcined phosphates.

Plant availability vs. chemical availability. The foregoing pot experiment has shown the relative availability of the phosphates under trial. It is of interest to compare this with the availability as determined by chemical methods. As equal quantities of total P_2O_5 were added to each pot, the

TABLE 3

Absorption of phosphoric acid by oats grown on sandy loam in pots receiving different amounts of various phosphates

FERTILIZERS	P_2O_5 ABSORBED			
	110 lbs. P_2O_5		220 lbs. P_2O_5	
		Net recovery		Net recovery
	per cent	*mgm.*	*per cent*	*mgm.*
Calcined phosphate A	0 59	15 9	0.71	28 2
Calcined phosphate B	0 50	11 0	0 71	25 0
Superphosphate	0 53	14 8	0 71	26 4
North African rock phosphate....	0 43	5 8	0 46	7 3
Tennessee brown-rock phosphate.	0 41	1 7	0 44	3 1
N and K	0 41		.	
None.. .	0 93		.	

TABLE 4

Summary of the comparative efficiency of certain phosphate fertilizers as measured by yield, composition of plant, and citrate solubility

FERTILIZERS	110 LBS. P_2O_5		220 LBS. P_2O_5		AVAILA-BILITY
	Dry weight	P_2O_5 recovered	Dry weight	P_2O_5 recovered	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Calcined phosphate A................. ..	99 0	107 2	102 4	106 7	105 2
Calcined phosphate B..	91 3	74 5	95 6	94 4	75 5
Superphosphate........	100 0	100 0	100 0	100.0	100 0
North African rock phosphate............	81 9	39 0	78 2	28 5	16 9
Tennessee brown-rock phosphate..........	65 5	11 2	63 8	11 8	6 8

various phosphates were rated on a percentage basis, the citrate-soluble P_2O_5 in superphosphate being given a value of 100 (table 4).

From the data in table 4 it is clear that the availability as shown by the rotation solubility in ammonium citrate agrees closely with that found by trial with plants.

SUMMARY

The availability of the new calcined phosphate was compared with that of superphosphate and of rock phosphates in experiments with plants. As

measured both by yields of oats and by amount of phosphoric acid recovered by the crop, the calcined phosphates were found to be equal to superphosphate and far superior to untreated rock phosphates.

These new phosphates have a high availability as measured also by their solubility in ammonium citrate, and a close agreement was found to exist between the chemical and the vegetation availabilities.

REFERENCES

(1) Aso, K. 1903 On the action of sodium fluoride upon plant life. *Bul. Col. Agr. Tokyo Univ.* 5: 187–196.

(2) Association of Official Agricultural Chemists 1930 Official and Tentative Methods of Analysis, ed. 3, Washington, D. C.

(3) BARTHOLOMEW, R. P. 1935 Fluorine, its effect on plant growth and its relation to the availability to plants of phosphorus in phosphate rock. *Soil Sci.* 40: 203.

(4) GAUTIER, A., AND CLAUSMAN, P. 1916 Le fluor dans le régne végétal. *Compt. Rend. Acad. Sci.* [Paris] 162: 105–112.

(5) HENDRICKS, S. B., HILL, W. L., JACOB, K. D., AND JEFFERSON, M. E. 1931 Structural characteristics of apatite-like substances and composition of phosphate rock and bone as determined from microscopical and X-ray diffraction examinations. *Indus. and Engin. Chem.* 23: 1413–1418.

(6) JACOB, K. D., BARTHOLOMEW, R. P., BROWN, B. E., PIERRE, W. H., REID, F. R., AND TIDMORE, J. W. 1935 Nutrient value of the phosphorus in calcined phosphate as determined by growth of plants in greenhouse experiments. *Jour. Agr. Res.* 50: 837–848.

(7) JACOB, K. D., BROWN, B. E., AND REID, F. R. 1935 Phosphate fertilizers prepared by treating phosphate rock with steam at high temperatures. *U. S. Dept. Agr. Yearbook* 1935: 279–282.

(8) JACOB, K. D., HILL, W. L., MARSHALL, H. L., AND REYNOLDS, D. S. 1933 The composition and distribution of phosphate rock, with special reference to the United States. U. S. Dept. Agr. Tech. Bul. 364.

(9) JACOB, K. D., AND REYNOLDS, D. S. 1928 The fluorine content of phosphate rock. *Jour. Assoc. Off. Agr. Chem.* 11: 237–250.

(10) JACOB, K. D., AND REYNOLDS, D. S. 1932 Occurrence of fluorine in natural phosphates. *Indus. and Engin. Chem.* 24: 86.

(11) JACOB, K. D., REYNOLDS, S. D., AND MARSHALL, H. L. 1936 Phosphate fertilizers by calcination process: Volatilization of fluorine from phosphate rock at high temperatures. Amer. Inst. Mining and Metall. Engin. Tech. Pub. 695; Class H, Indus. Min. Div. 37.

(12) MARSHALL, H. L., REYNOLDS, D. S., JACOB, K. D., AND RADER, L. F., JR. 1935 Phosphate fertilizers by calcination process; experiments with different phosphates. *Indus. and Engin. Chem.* 27: 205–209.

(13) MORSE, H. H. 1935 The toxic influence of fluorine in phosphatic fertilizers on the germination of corn. *Soil Sci.* 39: 177.

(14) REYNOLDS, D. S., AND JACOB, K. D. 1929 Ratio of fluorine to phosphoric acid in phosphate rock. *Indus. and Engin. Chem.* 21: 1253.

(15) REYNOLDS, D. S., JACOB, K. D., MARSHALL, H. L., AND RADER, L. F., JR. 1935 Phosphate fertilizers by calcination process: Factors affecting reaction between water vapor and Florida land pebble phosphate. *Indus. and Engin. Chem.* 27: 87.

(16) REYNOLDS, D. S., JACOB, K. D., AND RADER, L. F., JR. 1934 Phosphate fertilizers by calcination process: Action of silica and water vapor on phosphate rock. *Indus. and Engin. Chem.* 26: 406–412.

PLATE

PLATE 1

RELATIVE EFFICIENCY OF DIFFERENT PHOSPHATES

1. Calcined Phosphate A
5. Calcined Phosphate B
7. Superphosphate
11. North African Rock Phosphate
14. Tennessee Brown-Rock Phosphate
31. No Phosphate
F_2 No fertilizer

THE RELATIVE WEIGHT OF SHOOT AND ROOT OF DIFFERENT CROPS AND ITS AGRICULTURAL SIGNIFICANCE IN RELATION TO THE AMOUNT OF PHOSPHATE ADDED TO THE SOIL

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Persons who study the roots of agricultural crops generally attribute special significance to the relative weight of shoot and root because the shoot-root ratio is closely related to vital processes in the plant.

The shoot-root ratio differs greatly in different crops. That it also differs greatly in different varieties of the same crop was shown by Schneider (24) in a comparison of 88 varieties of oats, by Boonstra (1) with several strains of peas, and by Kulescha (15) with several strains of sugar cane. These investigators, independently of one another, determined that varieties with a high shoot-root ratio generally gave a larger yield than varieties with a low shoot-root ratio. Moreover, Boonstra (2) later showed that the amount of water and minerals absorbed per unit of root weight increased with the shoot-root ratio of the varieties.

The relative weight of shoot and root, however, is not constant for the same species or variety. It is a function of the age of the plant and of its environment. Pfeiffer and Simmermacher (21), Harris (11), and Boostra (1) have shown that the shoot-root ratio increases with the age of the plant; and Brenchley and Jackson (4) "found the shoot-root ratio of barley and wheat in pots practically constant or somewhat falling during the first nine to eleven weeks of growth, which was followed by a marked rise of the shoot-root ratio in the latter period of plant life."

Of the various environmental factors that affect the proportion of shoots to roots, the moisture content and the amount of available plant nutrients in the soil especially have been studied. It was shown by Tucker and Von Seelhorst (27) in 1898 with grain crops and later by Pfeiffer and Simmermacher (21) that the weight of both the shoots and the roots increases with increasing fertility of the soil, the former, however, to a greater extent; consequently the shoot-root ratio rises. The same authors found a rise in the shoot-root ratio when the moisture content of the soil was increased. Harris (11) made the same observation. He found also that a greater effect upon the shoot-root ratio was exerted by a change in water content than by a change in the amount of nutrients.

It seems evident that the decrease of the root weight in relation to the shoot

weight, when the content of water and nutrients in the soil is increased, is accompanied by a corresponding decrease in the water- and salt-absorbing surface of the roots, and that the decrease is overbalanced by the enhanced intake of water and nutrient salts per unit of absorbing root surface. Brenchley (3) suggested that "it seems as though the plant makes every endeavour to supply itself with adequate nutriment, and as if, when the food supply is low, it strives to make as much root growth as possible so as to offer the greatest absorbing surface for whatever nutriment may be available."

The actual absorbing surface should be considered a good basis for judging the efficiency of the root system, but unfortunately the determination of the total absorbing area of the root system necessitates a laborious procedure.[1]

From results obtained by Boonstra (2) with peas, it can be concluded that the ratio of dry weight of shoots to roots is a fairly satisfactory criterion for judging roughly the absorption efficiency of the root system of different plants of the same variety if the plants are grown on soils containing different amounts of water and nutrient salts. Boonstra found that the increase of the shoot-root ratio of the plants with increasing moisture content of the soil was associated with a rise in the amount of minerals absorbed by the plants per unit of root weight. When the peas were grown in Knop's nutrient solution of different concentrations, the shoot-root ratio increased with the concentration of the solution, and the quantity of minerals absorbed per gram of roots was also found to rise.

As it may be assumed that application of fertilizers results in an increased concentration of the soil solution, it may be concluded from the data obtained by Boonstra (2) that fertilizers added to the soil cause an increase of both the shoot-root ratio of the plants and the absorption of nutrient salts per unit of root mass. This may favor the growth of the above-ground parts and the yield of the crop.

Consequently, the shoot-root ratio may to a certain degree be considered as an indicator of the amount of available plant nutrients in the soil. The practical significance of this ratio, however, is rather limited, as collecting the roots in the field is very laborious and, because of the great extent of the root system, hardly practicable. Furthermore, the weight of the roots in relation to that of the shoots is dependent on other factors also, as has been indicated. On the other hand, the study of the shoot-root ratio in relation to the absorption of nutrient salts by the roots leads toward a better understanding of the significance of fertilizing and of its effect on plant growth than if this effect—as

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[1] Attempts to determine or to estimate the absorbing surface of the root system were made by Weaver and co-workers (31, 33), Dustman (6), and Nutman (18). A more profound study on the limitation of the absorbing area of the roots was made in the last 10 years by Popesco (22), Ursprung and Blum (30), and Sierp and Brewig (25), in whose papers the older literature is cited. The question as to how far the weight of the root system is indicative of root efficiency was critically discussed by Weaver and Himmel (32).

occurs in practice—is judged only by the growth of the aerial parts and the yield of the crop.

The question arises as to how great an increase of the shoot-root ratio of the crop is caused by the various nutrient salts necessary for plant growth.

It has long been known that nitrogen, under many conditions, causes an increase of the shoot-root ratio. In general, the weight of both shoot and root increases, the former to a greater extent, or only the shoot weight increases while the root weight remains constant or is lowered. Crist and Stout (5) found that the shoot-root ratio of lettuce on poor soil increased from 0.93 to 1.25 when 100 pounds $Ca(NO_3)_2$ an acre was added to the soil. When 300 and 600 pounds $Ca(NO_3)_2$ were applied, the shoot-root ratios rose still higher, to 1.58 and 1.64, respectively. Similar results with agricultural crops that were grown either in nutrient solutions or in soil were obtained by other investigators. I have often observed a rise in the shoot-root ratio of wheat in nutrient solutions with increasing nitrate concentrations. This rise was more striking among the lower concentrations. With higher nitrate concentrations, the shoot-root ratio increased little (8, 9). Turner (28) stated that "the increased ratio of tops to roots which results from increasing the amount of nitrate in the solution may be explained on the basis of the increased use of carbohydrates in the tops because the greater nitrogen supply makes for greater growth. This results in a decrease in the supply of carbohydrates for the roots, which may bring about an absolute or a relative reduction of root growth."

Only a few experimental data on the effect of potassium on the shoot-root ratio are available. Crist and Stout (5) found that the addition of 300 pounds KCl an acre to a soil deficient in potassium resulted in a rise of the shoot-root ratio of lettuce from 1.64 to 1.71, and, where more phosphate was available, from 1.95 to 2.03. From this result and from other data obtained, these authors concluded "that the top-root ratios show an unmistakable tendency to be continuously higher as more complete fertilization of the soil obtains."

As to the effect of phosphate, Turner (29) reported that "the ratios of top to root growth decrease as the phosphate concentration of the solution increases," and Miller (16, p. 102) wrote: "Nitrogenous fertilizers especially increase the growth of the tops as compared to the roots, while phosphorus as a rule increases the weight of the roots in greater proportion than the tops." Turner compared the growth of barley, wheat, and cotton in solutions with two widely differing phosphate concentrations. One of these solutions contained 0.021 gm. $CaHPO_4 \cdot 2H_2O$, 0.014 gm. $Mg_3(PO_4)_2 \cdot 4H_2O$, and 0.017 gm. KH_2PO_4 per liter nutrient solution, and the other solution contained 20 times as much phosphate. In the crops investigated by Turner, a lower shoot-root ratio was found in the solution with more phosphate than in the solution with less phosphate.

The minimum phosphate concentration necessary for maximum growth is very low—a fraction of 1 p.p.m. P_2O_5 (14, 20, 34). Below this critical concen-

tration, a slight variation in concentration causes great differences both in the growth of the plants and in the ratio of shoot- and root-weight. It is believed that with the lower concentrations the shoot-root ratio increases with the phosphate concentration, and that a decrease of the ratio does not occur until a certain concentration is exceeded. This view is supported by the fact that a rise of the shoot-root ratio has been observed after the application of phosphatic fertilizers to soil that is low in phosphate. The shoot-root ratio of lettuce grown on soil that received a supply of 300 pounds calcium mono-phosphate an acre rose from 1.71 to 2.03 (5). Similar observations have been made by others, for Russell states (23, p. 74) that "when phosphates are given [to cereals] the shoot increases more than the root, i.e. the ratio root-shoot is diminished."

A few years ago experiments were carried out at the Rijkslandbouwproefstation at Groningen with wheat (*Triticum vulgare* Vill), oats (*Avena sativa* L.), and horse beans (*Vicia faba* L.) that were grown in wooden boxes filled with sandy soil (heath soil) to which increasing amounts of phosphate had been added. The purpose of these experiments was to study the influence of the distribution of phosphate in the soil on the absorption of phosphate by the roots and on the development of the plants and their roots. The main results of this investigation will be published in a separate paper. Here only those results are given that bear upon the relation between the ratio of shoot- and root-weight and the amount of phosphate added to the soil. The phosphate in the form of calcium biphosphate was added to the various boxes in increasing quantities. In all experiments one culture series without phosphate was included. All the boxes received an equal and sufficient supply of nitrogen, potassium, and lime. As the relatively slight amount of lime contained in the calcium phosphate can hardly have had any influence on the crop, the effect of the phosphate was studied independently of that of other nutrient elements.

The plants of the different series were harvested on the same day, that is, at the beginning of flowering or somewhat later. In the soil that received no phosphate, flowering began one or two days later than in the other boxes; consequently, all plants were in nearly the same stage of development when harvested.

FIRST EXPERIMENT

Wheat plants were grown in boxes 20 cm. wide, 60 cm. long, and 100 cm. deep. A 60-cm. layer of diluvial sand free of phosphate (pH 5) was placed in each box, which was then filled with sandy soil. The sandy soil received 0.2 gm. $NaNO_3$, 0.17 gm. K_2SO_4, 0.1 gm. $MgSO_4$, 0.04 gm. $CuSO_4$, and 3.5 gm. $CaCO_3$ per kilogram air-dry soil. The boxes were dug into the earth, and in each box five wheat plants were grown. Ten boxes in all were prepared, differing only in the amount and the distribution of the phosphate applied to the 0–40 cm. layer.

No phosphate was added to the first box. To the second box was added a

quantity of phosphate insufficient for maximum growth of the plants. In the third and fourth boxes the 0–20 cm. layer (the surface layer) was likewise insufficiently supplied with phosphate, and the 20–40 cm. layer (the subsoil) was supplied with a sufficient and an excessive amount, respectively, of this fertilizer. The subsoil of boxes 5, 6, and 7 received the same treatments as did that of boxes 2, 3, and 4, but the surface layer in these boxes received an optimum amount of phosphate. In boxes 8, 9, and 10, excess phosphate was added to the surface soil, whereas the phosphate supply of the subsoil ranged from insufficient to abundant.

The amount and the distribution of the phosphate in the two layers were as follows:

BOX	P_2O_5 ADDED TO	
	Surface Soil	Subsoil
	gm.	*gm.*
1	0	0
2	0 1	0 1
3	0 1	0 4
4	0 1	0 7
5	0 4	0 1
6	0 4	0 4
7	0 4	0 7
8	0 7	0 1
9	0 7	0 4
10	0 7	0 7

A supply of 0.1 gm. P_2O_5 per kilogram dry soil was found in former experiments to be insufficient for good development of grain crops on these highly phosphate-binding soils; 0.4 gm. has proved to be nearly sufficient for the crop; and 0.7 gm. exceeds the need of the crop.

Shortly after the ears appeared, the plants were harvested, dried, and weighed. The roots were washed free from soil, after which they were likewise dried and weighed. The plants, exclusive of the roots, were analyzed for phosphate, after which the amount of phosphate absorbed by the plants and the amount absorbed per unit of root weight were calculated.[2]

In figure 1 the dry weight of the shoots and roots and the quantity of phosphate withdrawn from the soil by the plants are represented graphically.

In this figure the phosphate-deficiency of the soil used is clearly demonstrated by the low dry weight of the plants of box 1, to which no phosphate was added. As is shown by the figure, only a small amount of phosphate was absorbed by these plants, which developed typical symptoms of P-starvation. They showed bronze-purple leaves and a premature dying of the older leaves.

[2] The author is indebted to Dr. Th. van Itallie, chemist at the Rijkslandbouwproefstation, who supplied the analytical data.

Later the plants became pale and somewhat spindly. On the contrary, no P-starvation was observed in culture 2, although the plants of this culture were, from the beginning, inferior to those that received more phosphate. With the exception of culture 9, of which the majority of the plants were seriously attacked by the frit fly (*Oscinis frit* L.), the plants in all boxes supplied with phosphate were normal in appearance.

Table 1 gives the shoot-root ratios calculated from the dry weights of the shoots and the roots. The ratios are arranged in separate columns according to the thickness of the layer supplied with increasing amounts of phosphate
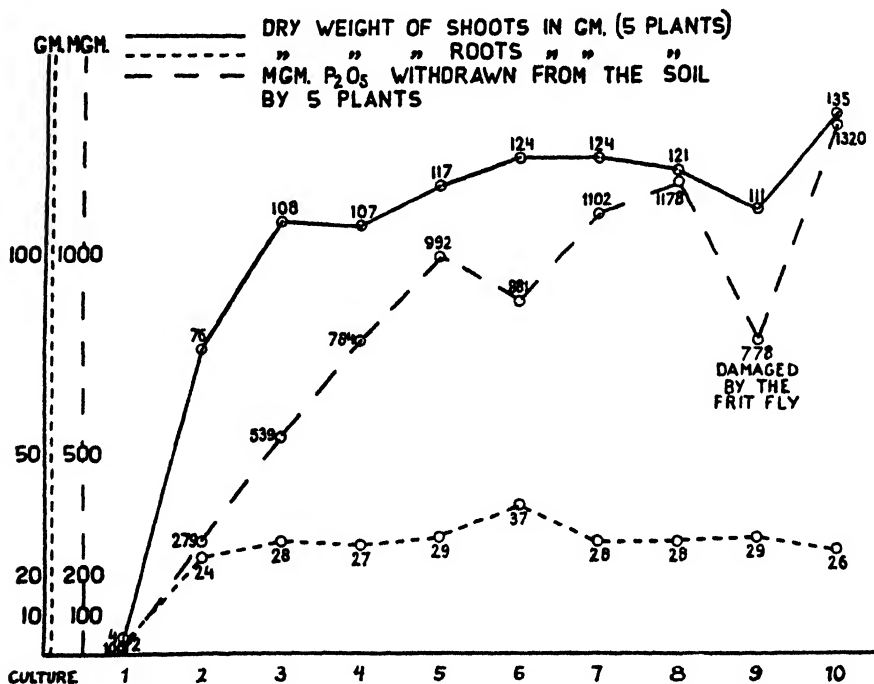
FIG. 1. DRY WEIGHTS OF SHOOTS AND ROOTS AND QUANTITIES OF PHOSPHATE WITHDRAWN FROM THE SOIL BY WHEAT PLANTS

and according to the phosphate supply in the subsoil. The shoot-root ratios in three of the columns show a rise with increasing applications of the phosphatic fertilizer. The figures in column 4 are exceptional in that their course deviates from that of the figures in other columns. The relatively low shoot-root ratio of culture 9 in this column is probably due to the damaging effect of the frit fly. For the deviation of culture 6 there is no plausible explanation.

Table 2 shows the relation between the quantity of phosphate absorbed and the shoot-root ratios of the plants; the dry weights of the shoots, roots, and entire plants; the absorption efficiency of the root system, i.e., the quantity of phosphate, in the shoot, absorbed from the soil per unit of root weight.

Table 2 shows that, with the exception of culture 6, an increase in the quantity of phosphate absorbed by the plants is accompanied by an increase in the ratio of shoot to root, which in turn is associated with an increase in the amount of phosphate absorbed per unit of root weight. In contrast with the other

TABLE 1

Relation between the shoot-root ratio of wheat plants and the amount of phosphate added to the soil

CULTURE NUMBER	SHOOT-ROOT RATIO			
	Increasing phosphate supply in 0–40 cm. layer	Increasing phosphate supply in 0–20 cm. layer		
		20–40 cm. layer insufficiently fertilized with phosphate	20–40 cm. layer sufficiently fertilized with phosphate	20–40 cm layer amply fertilized with phosphate
1	2 3			
2	3 2	3 2		
3			3 8	
4				4.1
5		4 0		
6	3 3		3 3	
7				4 4
8		4 4		
9			3 8	
10	5 2			5 2

TABLE 2

Relation between the amount of phosphate withdrawn from the soil, the dry weight and the shoot-root ratio of the plants, and the absorption efficiency of the root system of wheat, according to the amount and distribution of phosphate in the soil

CULTURE NUMBER	P_2O_5 WITHDRAWN FROM THE SOIL	DRY WEIGHT OF SHOOTS	DRY WEIGHT OF ROOTS	DRY WEIGHT OF ENTIRE PLANTS	SHOOT-ROOT RATIO	ABSORPTION EFFICIENCY OF THE ROOT SYSTEM (P_2O_5 ABSORBED PER GRAM ROOTS)
	mgm.	*gm.*	*gm.*	*gm.*		*mgm*
1	10	3 49	1 50	4 99	2 33	6 7
2	280	75 6	23 5	99 1	3 22	11 9
3	540	107 9	28 3	136 2	3 81	19 0
9	776	110 8	29 0	139 8	3 82	26 8
4	783	107 3	26 5	133 8	4 05	29 5
6	881	124 1	37 4	161 5	3 32	23 6
5	993	116 8	29 0	145 8	4 03	34 2
7	1103	123 9	28.4	152 3	4 36	38 8
8	1177	121 3	27 6	148 9	4 39	42 6
10	1321	134 8	26 0	160 8	5 18	50 8

cultures, culture 6 combines a high plant weight with a comparatively low shoot-root ratio and a low rate of absorption per gram of roots.

One might conclude from table 2 that, with the exception of culture 6, there is a positive correlation between the total amount of phosphate absorbed by

the plants, the shoot-root ratio of the plants, and the amount of phosphate absorbed per unit of root weight.

The dry weights of the roots increased with the supply of phosphatic fertilizer in cultures 1, 2, and 3 (table 2 and fig. 1) but, with the exception of culture 6, did not seem to increase further with a more liberal supply of phosphate.

Table 2 shows that, in general, the quantity of phosphate absorbed increased with the total amount of phosphate added to the soil (0–40 cm.), but several deviations may be noted. It seems evident that the phosphate in the surface soil resulted in a greater absorption of phosphate by the plants than did an equal supply of phosphate in the subsoil. Culture 5, which received a fair amount of phosphate in the surface layer, withdrew considerably more phosphate from the soil than did culture 3, which received an equal amount of phosphate that was more concentrated in the subsoil. Cultures 4, 6, and 8
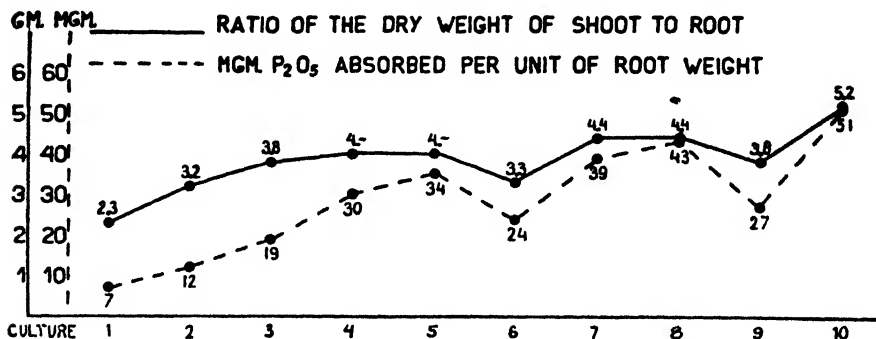
FIG. 2. SHOOT-ROOT RATIOS AND QUANTITIES OF PHOSPHATE ABSORBED PER UNIT OF ROOT WEIGHT OF WHEAT PLANTS

also showed a rise in the intake of phosphate with an increasing supply in the surface soil, although the total amount of the phosphatic fertilizer was equal in these three cultures. A striking deviation is shown by culture 9, in which the quantity of phosphate absorbed was much lower than would be expected from a culture that received so much phosphate. This deviation is probably due to the injurious effect of the frit fly.

It may be noted also that the plants of culture 10 yielded a higher dry weight than one would expect from the graph of the shoot weights in figure 1. Indeed, this curve shows that the maximum growth had already been reached with the cultures that had received 0.4 gm. P_2O_5 per kilogram soil in the 0–20 cm. layer and a liberal phosphate supply in the subsoil. As has been found in other experiments, a quantity of fertilizer which is supra-optimal in respect to plant growth may produce a more abundant absorption of nutrients by the plant roots but does not increase the growth of the crop. It is highly probable that the increase in weight of culture 10 must be attributed to the fact that

the plants of this culture, which was the last one in the row, were more exposed to light than were the other cultures.   As is indicated in table 2, the roots of culture 10 were highly efficient in the absorption of phosphate.   The considerable increase in the shoot-root ratio and in the dry weight of the plants of this culture shows how much these plants have been favored by the increased activity of the root system as well as by the greater amount of light.

In brief, table 2 clearly shows that a rise in the shoot-root ratio is generally correlated with an increase in the absorption efficiency of the root system. This is also clearly indicated in figure 2, in which the shoot-root ratio and the amount of phosphate absorbed per gram roots are represented graphically in relation to the supply of phosphate in the soil.

This figure shows that the lowering of the shoot-root ratio produced by lack of available phosphate in the soil (as shown by cultures 3, 2, and 1) or by other causes (as in cultures 6 and 9) is associated with a decrease in the amount of phosphate absorbed per unit of root weight.   Conversely, a rise in the shoot-root ratio roughly coincides with an increase of the phosphate absorption per gram roots.

In general it may be concluded from the results of this experiment that, insofar as the conditions were favorable for the absorption of phosphate by the plants, both the shoot-root ratios of the plants and the absorption efficiency of the root systems were raised.

SECOND EXPERIMENT

Results similar to those for wheat were obtained with horse beans, which were also grown in deep boxes.   The 0–20 cm. layer consisted of the same sandy soil as that used in the first experiment and rested on a layer of diluvial sand, the pH of which was 5.   The same fertilizers and the same quantity of lime were applied as in the previous experiment.   The phosphate was supplied in the form of calcium biphosphate to depths of 5, 10, and 20 cm. and in increasing amounts.

In each box were grown four plants, which showed a marked uniformity of growth.   The plants were harvested at early flowering.   The roots were uncovered by washing away the soil, after which they were dried and weighed. At harvesting it appeared that the root systems, in contrast to those of the wheat, were almost exclusively confined to the 0–20 cm. layer, only a few roots having penetrated into the underlying acid sand layer.   The percentage of phosphate on a dry-weight basis of both shoots and roots was determined. The values obtained were used for calculating the total amount of phosphate absorbed by the plants during the whole period of life.

The purpose of this experiment was to study the effect of the amount and distribution of phosphate on root development.   Here only the relationship between the phosphate supply and the shoot-root ratio of the plants will be considered.

Table 3 shows the relation of the shoot-root ratios of the plants, the dry

weights of shoots and roots, the total amounts of phosphate absorbed by the plants, and the amounts of phosphate absorbed per gram of roots to the amount of phosphate added to the soil. The figures are arranged in three groups according to the depth to which the phosphate was applied.

The table shows that in the box which received no phosphate the plants returned phosphate to the soil. At harvest these plants contained less phosphate than did the seed that was planted. Soon after their appearance above ground these plants began to lag behind the others, and after a few weeks they showed symptoms of phosphorus-starvation. In the box that was supplied to a depth of 20 cm. with 0.1 gm. P_2O_5 per kilogram soil (culture 4) P-starva-

TABLE 3

Relation between the amount of phosphate added to the soil, the dry weight and the shoot-root ratio of the plants, the amount of phosphate withdrawn from the soil, and the absorption efficiency of the root system of horse beans

DEPTH TO WHICH PHOSPHATE WAS APPLIED	CULTURE NUMBER	P_2O_5 SUPPLY*				DRY WEIGHT OF SHOOTS	DRY WEIGHT OF ROOTS	DRY WEIGHT OF ENTIRE PLANTS	SHOOT-ROOT RATIO	P_2O_5 ABSORBED BY PLANTS	ABSORPTION EFFICIENCY OF THE ROOT SYSTEM (P_2O_5 ABSORBED PER GRAM ROOTS)
		0-5 cm. layer	5-10 cm. layer	10-15 cm. layer	15-20 cm. layer						
cm.		gm.	gm.	gm.	gm.	gm.	gm.	gm.		mgm.	mgm.
0–20	1	0	0	0	0	8 0	3 2	11 2	2 5	−13	−4 1
	4	0 1	0 1	0 1	0.1	11 2	5 1	16 3	2 2	+11	+2 2
	7	0.2	0 2	0.2	0 2	27 0	9 0	36 0	3 0	+143	+15 9
	8	0 4	0.4	0 4	0 4	31.9	7 7	39 6	4.1	+261	+33.9
0–10	1	0	0	0	0	8 0	3 2	11.2	2 5	−13	−4 1
	3	0.2	0 2	0	0	17 7	5 5	23 2	3 2	+70	+12.7
	6	0 4	0.4	0	0	30 5	8 6	39 1	3 5	+238	+27 7
0–5	1	0	0	0	0	8 0	3 2	11.2	2 5	−13	−4.1
	2	0.4	0	0	0	15.5	6 0	21.5	2.6	+79	+13 2
	5	0.8	0	0	0	26 3	8.2	34.5	3.2	+252	+30.7

* Per kilogram air-dry soil.

tion was also observed, but not until the end of the growth period. Apparently these plants gave off P_2O_5 during the later stages of growth, thus depressing the total P_2O_5-absorption for the whole period of growth to only 11 mgm. Although these plants grew better than did those in the box without phosphate, they were decidedly inferior to the cultures that received more phosphate and to the cultures that received the same amount of phosphate concentrated in a thinner layer.

Table 3 shows that, with one exception, the shoot-root ratio was increased with increasing amounts of phosphate added to corresponding layers of the soil. Furthermore, this table shows that there is a distinct positive correlation

between the phosphate supply, the dry weight of the plants, the amount of phosphate withdrawn from the soil by the plants, and the amount of phosphate absorbed per gram of roots. It may be noted that an increase in the supply of phosphate in the 0–20 cm. layer from 0.2 to 0.4 gm. P_2O_5 per kilogram soil (cultures 7 and 8) resulted in a considerable increase of the shoot-root ratio, of the rate of absorption of phosphate by the roots, and of the amount of phosphate absorbed per gram roots, whereas the dry weight of the plants rose only slightly. Evidently, 0.2 gm. P_2O_5 per kilogram of soil was nearly the optimum supply, and 0.4 gm. P_2O_5 apparently gave rise to a superfluous absorption of phosphorus by the plant roots.

DISCUSSION OF RESULTS OF FIRST AND SECOND EXPERIMENTS

It was found that application of phosphatic fertilizers increased the shoot-root ratios of both wheat and horse beans. In both experiments this increase was associated with a greater quantity of phosphate absorbed per unit of root weight.

In the second experiment the absolute root weight generally increased with the supply of phosphate in the soil, but in the first experiment the increase was observed, with one exception, only with low phosphate dressings. This tendency of the root weight to increase with increasing quantities of phosphate is in agreement with the result obtained by others that phosphorus promotes root development (23, p. 74). A greater root mass was associated in our experiments with a rise in the amount of phosphate absorbed per gram of roots, and as a consequence the amount of phosphate absorbed by the plants increased considerably.

That liberal application of phosphate may cause a reduction of the root mass was demonstrated with the horse beans when the phosphate supply rose from 0.2 to 0.4 gm. P_2O_5 per kilogram of soil in the 0–20 cm. layer. Because of the large increase in the quantity of phosphate absorbed per gram roots, the total amount of phosphate taken up by the plants increased considerably, in spite of the reduction of the root weight (table 3, cultures 7 and 8). The root weights of the wheat plants in the first experiment, in general, remained fairly constant in the range of higher phosphate supplies. Nevertheless the total amount of phosphate absorbed by these plants rose with increasing phosphate supply as a consequence of the increased absorption efficiency of the root system, as is shown in table 2.

The rise in the shoot-root ratio with increasing phosphate supply was accompanied, in general, by an increase in shoot development, which promoted carbon assimilation and growth of the crop. The photosynthetic activity, however, depends on the total area of the assimilating parts and on the carbon assimilation per unit of area. Gregory (10), who studied the effect of mineral nutrition on the rate of growth and assimilation, states that "over a short range near zero concentration the increase in [phosphate] concentration results in a higher leaf growth rate as well as higher assimilation efficiency"; and Russell

says (23, p. 77), "Later doses [of phosphate], however, increase only the rate of growth. There is thus a point of inflexion in the curve showing the relationship between amount of phosphate absorbed and amount of growth."

In brief, phosphate produces an increase in the shoot-root ratio and in the development of the aerial parts, which is associated with a higher rate of absorption of phosphate by the roots. The carbon-phosphorus ratio apparently is favorable for the growth of the plant under the prevailing phosphate conditions. The significance of this ratio for the plant becomes clear if we bear in mind that phosphate is essential for the formation of lecithin, nucleoproteins, and other organic compounds which contain phosphorus. It is evident, however, that this teleological viewpoint offers no explanation of the phenomena observed.

The results obtained relate to plants that were grown in soil. Inasmuch as it may be assumed that application of phosphate increases the phosphate concentration of the soil solution surrounding the roots, it seems evident that in nutrient solutions plants will show a similar response to the phosphate concentration of the solution. Turner, however, found a decrease of the shoot-root ratio with more phosphate in nutrient solutions containing two different phosphate concentrations. It has already been suggested that the decrease of the shoot-root ratio in Turner's solutions was due to the high phosphate concentrations used by him. If this is correct, then the shoot-root ratio may also be expected to decrease in soil which is supplied with an excess of phosphate. This phenomenon was actually observed in the third experiment, a description of which follows.

THIRD EXPERIMENT

The object of this experiment was to determine the morphological structure and the phosphate-absorbing capacity of the root system when the phosphate in the soil is concentrated in vertical layers alternating with layers poor in phosphate. Besides cultures receiving localized applications of phosphate, plants were grown in boxes in which increasing amounts of phosphate were distributed uniformly throughout the soil. It is more especially the results with these latter cultures which are discussed in this paper. In the cultures with localized applications of phosphate, complications occurred which will be discussed in detail in a later paper.

In this experiment oats were grown in small boxes, 20 cm. deep, 10 cm. long, and 10 cm. wide, two plants in each box. Each culture consisted of five boxes, i.e., 10 plants, which showed a rather uniform growth. The moisture content of the soil was kept fairly constant by repeatedly weighing the cultures and by supplying as much water as was lost by transpiration. Heath soil was used for this experiment. All boxes received the same amount of nitrogen, potassium, and lime, which was sufficient for maximum growth of the crop.

In one of the series no phosphate was added. The other series were given calcium biphosphate in increasing amounts, 0.1, 0.2, 0.4, and 0.8 gm. P_2O_5 per

kilogram soil respectively. When harvested at the beginning of ripening, the plants were treated in the same way as in the previous experiments. The shoots as well as the roots were analyzed for phosphate.

The plants in the boxes without phosphate remained very small and at an early stage showed symptoms of phosphate starvation. These plants, however, in contrast with the horse beans in the second experiment, were found to have succeeded in withdrawing a little phosphate from the soil. With increasing supply of phosphate the growth increased, until maximum growth was reached at 0.2 gm. P_2O_5 per kilogram soil. The plants in the boxes with 0.4 and 0.8 gm. P_2O_5, which at first seemed superior to the others, began after a time to lag behind in growth. The poor growth was especially striking in the boxes supplied with 0.8 gm. P_2O_5. The plants grew paler and seemed to lack nitrogen or something else, which apparently was caused by the large amount of phosphate added to the soil.

TABLE 4

Relation between the amount of phosphate added to the soil, the dry weight and the shoot-root ratio of the plants, the amount of phosphate withdrawn from the soil, and the absorption efficiency of the root system of oats

CULTURE NUMBER	P_2O_5 SUPPLY[*] IN SOIL	DRY WEIGHT OF SHOOTS	DRY WEIGHT OF ROOTS	DRY WEIGHT OF ENTIRE PLANTS	SHOOT-ROOT RATIO	P_2O_5 ABSORBED BY PLANTS	ABSORPTION EFFICIENCY OF THE ROOT SYSTEM (P_2O_5 ABSORBED PER GRAM ROOTS)
	gm.	*gm.*	*gm*	*gm.*		*mgm.*	*mgm*
1	0	3 515	0 865	4 38	4 1	10	11 6
2	0 1	67 72	5 85	73 57	11 6	216	36 9
3	0 2	84 67	7 43	92 10	11 4	315	42 4
4	0 4	82 07	7 65	89 72	10 7	568	74 2
5	0 8	74 0	9 78	83 78	7 6	1035	105 9

[*] Per kilogram air-dry soil.

In table 4 the shoot-root ratios of the plants and the amounts of phosphate drawn from the soil are given, as well as the values from which these figures were calculated.

The weights of the entire plants given in the table support the conclusion, drawn from superficial examination of the growing plants, that growth reached its maximum at about 0.2 gm. P_2O_5. Below this optimum there is a distinct decrease in the plant weight with a decreasing supply of phosphate, this decrease being associated with a decline in the amount of P_2O_5 absorbed per gram roots and in the total amount of phosphate absorbed by the plants. The table shows also that the shoot-root ratio of the plants grown on soil without phosphate is much lower than that of the plants grown on soil to which phosphate was added. These results agree with those obtained with wheat and beans in the previous experiments, in which a positive correlation between the phosphate supply, the shoot-root ratio of the crop, the absorption efficiency of

the roots, and the total amount of phosphate absorbed by the plants was found.

In the experiment with oats, however, above the optimum, the shoot weight, plant weight, and the shoot-root ratio decreased with increasing phosphate supply (table 4); in fact, the decrease in the shoot-root ratio seems to have begun below the growth optimum. These decreases are accompanied by striking increases in the amount of phosphate absorbed by the plants and in the amount of phosphate absorbed per unit of root weight. This result differs from that in the range of lower phosphate supplies and from that in the previous experiments, in that a decrease of the shoot-root ratio was generally associated with a reduction of the amount of phosphate absorbed by the plants (table 3 and fig. 2) and no decrease in plant weight was observed with phosphate supply that exceeded the optimum.

The discrepancy between the results obtained in this experiment and those in the previous experiments may be explained, perhaps, by the fact that the plants in the present experiment were grown in small shallow boxes, the entire soil of which was supplied with phosphate, whereas the wheat and bean plants of previous experiments grew in large deep boxes, in which a thick layer of poor sand under the more or less fertile surface soil was available for the roots. This idea is supported by the following phenomenon. In the experiment with oats, besides the cultures described, plants were grown in boxes in which only one half of the soil was supplied with increasing quantities of phosphate. The plants of these series, which are not described in this paper, showed no decrease in growth with increasing amounts of phosphate in the soil. On the contrary, these cultures developed vigorously without showing a trace of paleness and sent out a number of roots into the low-phosphate layer. A high plant weight and no decrease in the shoot-root ratio were observed with these cultures, to which a large amount of phosphate was locally supplied.[3]

The effect of a liberal supply and a uniform distribution of phosphate in this experiment is similar to that in the water-culture experiment of Turner (29), in which the phosphate likewise caused a reduction of the shoot-root ratio. In both experiments the roots developed in vessels in which the nutrients were uniformly distributed. In such a medium, plant roots are more sensitive to high concentrations than they are in the field, where a larger soil volume is available for the roots and where a more or less fertile surface layer rests on a subsoil which is generally deficient in phosphate.[4]

Sommer (26) recently studied the influence of the phosphate concentration of nutrient solutions on the root development of wheat, buckwheat, peas,

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[3] For further particulars on this experiment and on the influence of a localized application of phosphate on the growth of the plants and their root systems, the reader is referred to a preliminary publication (9).

[4] For information on the availability of phosphate in the subsoil to the plant, the reader is referred to the publications by Honcamp and Steinfatt (13), Engels (7), and Hetterschij (12).

tomatoes, maize, and cotton. The P_2O_5 concentrations, which in her experiments ranged from 0.1 to 3.2 p.p.m., were much lower than those in Turner's solutions. Within the range of concentrations employed by Sommer all crops showed an increase of the shoot-root ratio with increasing phosphate concentration. She suggested that the divergent result obtained by Turner (29) was due to the much higher phosphate concentrations used by him in his solution cultures.

We may conclude, therefore, that below a certain limit of phosphate the shoot-root ratio increases with increasing phosphate supply and that above that limit this ratio decreases.

When grown in soil, plants will probably show a similar response to the phosphate supply. It may be noted, however, that in several fertile soils of the United States Pierre and Parker (19) found the P_2O_5 concentration of the soil solution to be far less than 1 p.p.m. Similar values were obtained by Von Wrangell (34) in German soils and by Neeb (17) in Java soils.

From these data it is apparent that the phosphate concentration of most soil solutions is considerably lower than the concentrations used by Turner in his solution cultures. On the contrary, the concentrations of Sommer's solutions correspond more closely to the P_2O_5 concentration commonly found in the soil solution. On this account it may be expected that application of phosphate to soil which is deficient in phosphorus will cause an increase in the shoot-root ratio of the crop, as was actually found in our experiments with wheat and horse beans. In the experiment with oats the shoot-root ratio decreased, though only under rather unnatural conditions and with phosphate supplies that were higher than those required for maximum growth of the crop.

It may be concluded from the experiments described in this paper that application of phosphatic fertilizers, as a rule, increases the growth of the shoots to a greater extent than that of the root systems, thus increasing the shoot-root ratio of the plants.

SUMMARY

Wheat, horse beans, and oats were grown in wooden boxes containing sandy soil low in phosphate, to which increasing amounts of phosphate ($CaHPO_4$) were added.

The purpose of these experiments was to study the effect of the distribution of the phosphate in the soil on the development of the root system. In the foregoing pages only the results of the relationship between the phosphate supply and the ratio of the weight of shoots to roots are discussed.

There is some diversity of opinion as to the effect of phosphate on the shoot-root ratio of plants, and few experimental data are available in the literature.

In our experiments it was found that the shoot-root ratio, in general, increased with increasing phosphate supply. The weights of both the shoot and the root increased with the amount of phosphate, the shoot weight to a greater

extent than the root weight.   With large applications of phosphate the root weight remained constant or was lowered, and the shoot-root ratio continued to increase.

In the experiment with oats, however, a decrease of the shoot-root ratio was observed with increasing phosphate supplies, but only when the plants were grown in small boxes in which the phosphate was uniformly distributed and when excessive amounts of phosphate were supplied.

The data obtained by Sommer (26) with solution cultures and by the author with soil cultures seem to be sufficient evidence that the application of phosphate, in general, increases the shoot-root ratio of the crop.   Consequently, no essential difference exists between the effect of nitrogen and the effect of phosphorus on the shoot-root ratio.

With increasing phosphate supply the increase of the shoot-root ratio was associated with a rise in the amount of phosphate absorbed per unit of root weight.   The latter rose to such a degree that the total amount of phosphate absorbed by the plants increased considerably in spite of the relative or the absolute diminution of the root weight.   In other words, the reduction of the root weight was overbalanced by the enhanced intake of phosphate by the roots.

The rate of photosynthesis was likewise increased, though more especially in the range of lower phosphate supplies.   This was indicated by the increasing weights of the plants when greater amounts of phosphorus were available in the soil.   A relationship exists between the shoot-root ratio and the rate of photosynthesis.   This rate rises when the shoot-root ratio increases, as the latter is accompanied by a more extensive development of the shoot, i.e., by an increase of the assimilating surface of the plant.

The agricultural significance of the shoot-root ratio is theoretical.   To a certain degree this ratio may be considered as an indicator of the fertility of the soil.   As compared to the study of the above-ground parts and crop yields, the determination of the shoot-root ratio in relation to the absorption of nutrients by the roots leads to a better understanding of the significance of fertilizing practices and of their effect upon plant growth.

REFERENCES

(1) BOONSTRA, A. E. H. R.   1931   Root systems of seven varieties of peas grown under similar cultural conditions.   *Meded. Landbouwhoogesch.* [Wageningen]   35: 1–55.

(2) BOONSTRA, A. E. H. R.   1934   Physiologisch Onderzoek ten Dienste der Plantenveredeling.   Diss. Wageningen.

(3) BRENCHLEY, W. E.   1916   The effect of the concentration of the nutrient solution on the growth of barley and wheat in water cultures.   *Ann. Bot.* [London] 30: 77–90.

(4) BRENCHLEY, W. E., AND JACKSON, V. G.   1921   Root development in barley and wheat under different conditions of growth.   *Ann. Bot.* [London] 35: 533–556.

(5) CRIST, J. W., AND STOUT, G. J.   1929   Relation between top and root size in herbaceous plants.   *Plant Physiol.* 4: 63–85.

(6) DUSTMAN, R. B. 1925 Inherent factors related to absorption of mineral elements by plants. *Bot. Gaz.* 79: 233–264.

(7) ENGELS, O. 1931 Untersuchungen über den Löslichkeitsgrad der Phosphorsäure (relative Löslichkeit) in der Ackerkrume und dem Untergrund verschiedener Bodenarten. *Ztschr. Pflanzenernähr, Düngung, u. Bodenk.* 10: 181–187.

(8) GOEDEWAAGEN, M. A. J. 1933 De Invloed van de Nitraatconcentratie der Voedingsoplossing op den Groei van Tarweplanten. *Diss. Utrecht.*

(9) GOEDEWAAGEN, M. A. J. 1933 Het wortelstelsel der graanplanten bij ongelijke verdeeling der meststoffen in den grond. *Dept. Binnenland. Zaken en Landb.* [*Netherlands*], *Verslag. Landbouwk. Onderzoek. Rijkslandbouw Proefsta.* 39A: 343–362.

(10) GREGORY, F. G. 1931 The mineral nutrition of barley. *Proc. Fifth Internatl. Bot. Cong.* 1930: 440.

(11) HARRIS, F. S. 1914 The effect of soil moisture, plant food and age on the ratio of tops to roots in plants. *Jour. Amer. Soc. Agron.* 6: 65–75 [Cited by Miller (16)].

(12) HETTERSCHIJ, C. W. G. 1935 Die Verteilung der Phosphorsäure im Boden. *Die Phosphorsäure* 5: 215–230.

(13) HONCAMP, F., AND STEINFATT, F. 1927 Ueber den Nährstoffgehalt von Ackerkrume und Untergrund und seine Bedeutung fur die Untersuchung der Böden. *Ztschr. Pflanzenernähr., Dungung, u. Bodenk.* 6: 21–27.

(14) HONERT, T. H. VAN DEN 1933 Onderzoekingen over de voedingsphysiologie van het suikerriet. 2e Bijdrage. Proeven over phosphaatopname. *Meded. Proefsta. Java-Suikerindus* 23: 1119–1156.

(15) KULESCHA, M. 1931 Oriënteerend onderzoek over de ontwikkeling van het wortelstelsel bij 2878 POJ in verband met de grondsoort. *Meded. Proefsta. Java-Suikerindus.* 8: 317–358.

(16) MILLER, E. C. 1931 Plant Physiology, ed. 1. McGraw-Hill Book Co., New York.

(17) NEEB, G. A. 1933 Een onderzoek over de phosphaatbehoefte der Javasuikerrietgronden. *Meded. Proefsta. Java-Suikerindus.* 41: 1031–1086.

(18) NUTMAN, F. J. 1934 The root-system of *Coffea arabica*: III. The spatial distribution of the absorbing area of the root. *Empire Jour. Exp. Agr.* 2: 293–302.

(19) PIERRE, W. H., AND PARKER, F. W. 1927 Soil phosphorus studies: II. The concentration of organic and inorganic phosphorus in the soil solution and soil extracts and the availability of the organic phosphorus to plants. *Soil Sci.* 24: 119–128.

(20) PARKER, F. W. 1927 Soil phosphorus studies: III. Plant growth and the absorption of phosphorus from culture solutions of different phosphate concentrations. *Soil Sci.* 24: 129–146.

(21) PFEIFFER, TH., AND SIMMERMACHER, W. 1917 Über den Einfluss des Standraumes bezw. verschiedener Bodenarten auf die Wurzelmasse der Pflanzen. *Landw. Vers. Sta.* 90: 291–320.

(22) POPESCO, ST. 1927 Recherches sur la région absorbante de la racine. *Compt. Rend. Soc. Biol.* [Paris]. 96: 1031–1033.

(23) RUSSELL, E. JOHN 1932 Soil Conditions and Plant Growth, ed. 6. Longmans, New York.

(24) SCHNEIDER, G. 1912 Vegetationsversuche mit 88 Hafersorten. *Landw. Jahrb.* 42: 767–822.

(25) SIERP, H., AND BREWIG, A 1935 Quantitative Untersuchungen über die Wasserabsorptionszone der Wurzeln. *Jahrb. Wiss. Bot.* 82: 99–122.

(26) SOMMER, ANNA L. 1936 The relationship of the phosphate concentration of solution cultures to the type and size of root systems and the time of maturity of certain plants. *Jour. Agr. Res.* 52: 133–148.

(27) TUCKER, M., AND SEELHORST, C. VON   1898   Der Einfluss, welchen der Wassergehalt und der Reichtum des Bodens auf die Ausbildung der Wurzeln und der oberirdischen Organe der Haferpflanze ausüben. *Jour. Landw.* 46: 52–63.

(28) TURNER, TH. W.   1922   Studies of the mechanism of the physiological effects of certain mineral salts in altering the ratio of top growth to root growth in seed plants. *Amer. Jour. Bot.* 9: 415–445.

(29) TURNER, TH. W.   1929   Effect of mineral nutrients upon seed plants. II. Phosphates. *Bot. Gaz.* 88: 85–95.

(30) URSPRUNG, A., AND BLUM, G.   1928   Ueber die Lage der Wasserabsorptionszone in der Wurzel. *Beibl. Vierteljahrsschrift Naturforsch. Gesell. Zürich,* 73: 162–189.

(31) WEAVER, J. E.   1925   Investigations on the root habits of plants. *Amer. Jour. Bot.* 12: 502–509.

(32) WEAVER, J. E., AND HIMMEL, W. J.   1929   Relation between the development of root system and shoot under long- and short-day illumination. *Plant Physiol.* 4: 435–457.

(33) WEAVER, J. E., KRAMER, J., AND REED, M.   1924   Development of root and shoot of winter wheat under field environment. *Ecology* 5: 26–50.

(34) WRANGELL, M. VON   1930   Die Bestimmung der pflanzenzugänglichen Nährstoffe des Bodens. *Landw. Jahrb.* 71: 149–169.

NITROGEN METABOLISM OF SOYBEANS IN RELATION TO THE SYMBIOTIC NITROGEN FIXATION PROCESS[1]

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Attempts to determine the form in which atmospheric nitrogen is fixed symbiotically by association of rhizobia and host plant should include a study of the nitrogen metabolism of the plant. A study of the nitrogen conversions in plants receiving their nitrogen only through the symbiotic fixation process compared with the "normal" metabolism in plants supplied with nitrogen in a combined form might lead to a better understanding of certain aspects of the mechanism of symbiotic nitrogen fixation. Present knowledge of the mechanism is so limited that no procedure that offers possible illumination of the biological process should be neglected, even though obvious difficulties will be encountered in obtaining and interpreting the data.

The mechanism of "normal" nitrogen metabolism of plants is as yet imperfectly understood, although many valuable observations have been made (6, 7). For this reason comparative studies on nitrogen metabolism as related to the symbiotic fixation process must necessarily be more or less of the nature of empirical observations. The experiments reported in this paper are an attempt to determine whether any gross differences exist in the nitrogen metabolism of soybean plants grown under conditions comparable except for the source of nitrogen, i.e., combined nitrogen *versus* the unknown products of symbiotic nitrogen fixation.

Differences in the nitrogenous components may arise from several sources other than the type of nitrogen available. Chief among these are light intensity, length of day, humidity, temperature, and physiological balance, e.g., relation of carbohydrate and nitrogen. Because all these complicating factors are difficult to control in large-scale experiments, differences noted in the nitrogen components of the inoculated and the nitrate-fed plants may not be directly attributable to the fixation process. In order to determine whether the method is promising, however, it is important to know whether such differences do exist.

As the interpretation of complex nitrogen metabolism studies is difficult and shows little agreement, a brief review of the basis for the experiments

reported seems desirable. As the leguminous plant grows almost equally well (12) upon the products of symbiotic nitrogen fixation as upon the combined forms of nitrogen (NH_4NO_3), it is reasonable to assume that many of the plant processes are independent of the source of nitrogen; that is, once the nitrogen has become translocatory, subsequent metabolism should be identical in the two types of plants. In this connection it should be noted that in the soybean, with the possible exception of the young seedling, amides do not constitute the sole form of nitrogen concerned in transport functions (16).

If the conversion of the source of nitrogen into the more stable forms used for transport or for protein synthesis involves compounds that are dependent on the particular nitrogen source, detection of these compounds should be facilitated by analysis of only the soluble nitrogen fraction rather than the entire tissue. For this reason and also to simplify the study, the investigations were confined to detailed examination of the non-coagulable soluble nitrogen fraction of the plant. On the other hand, in spite of the large increase in the number of analyses required, it was believed necessary to analyze separately the various organs of the plants—roots, stems, leaves, and nodules. Unless this is done a significant difference in one part of the plant may be masked by an equally significant but opposing difference in another part. Likewise, interpretation of the data is less difficult if the analyses are separate for the different organs.

It may be argued that in our present state of ignorance with respect to plant metabolism and because of the lack of reliable methods, attempts to elucidate the nitrogen fixation mechanism by this approach are foredoomed to failure. Nevertheless, it is felt that even with this handicap some progress can be made; moreover, this approach affords another means of investigation into a problem for which means are all too few. It is granted that information will necessarily be indirect and conclusions tentative, but combined with the observations from other methods of approach, the results may be of aid in deciding between alternative mechanisms or even may be suggestive of other possibilities to be further investigated.

METHODS

Three series of Manchu soybeans were used in each experiment, as follows:

(A) *Inoculated.* The plants were inoculated with a mixture of three efficient strains of *Rh. japonicum* from the Wisconsin collection, but no forms of combined nitrogen were added.

(B) *High-nitrogen.* The plants were not inoculated, but fixed forms of nitrogen were added periodically. No nodules were present.

(C) *Low-nitrogen.* The plants were not inoculated but were furnished combined nitrogen in exactly half the amount given the high-nitrogen series. Nodules were absent.

Attempts were made to add nitrogen at such a rate that the inoculated plants would have nitrogen contents between those of the two combined nitrogen series. Ammonium nitrate (NH_4NO_3) was used so that neither cation or anion would be left unused in the assimilation process and thus introduce further variables.

The plants were grown in sterilized pit sand and given a modified Crone's nitrogen-free nutrient solution. The methods of plant culture have been described elsewhere (8, 9, 11, 12, 13). The dry weight, total nitrogen, and nitrate addition curves of the experiments here reported have been discussed by Umbreit and Fred (12) as examples of the influence on nitrogen fixation of optimum and near-optimum conditions of the carbohydrate-nitrogen relation in the soybean. For convenience the experiment numbers used in the previous report are used here.

The analysis of the soluble portion of the fresh tissue for its nitrogen components offers considerable difficulty that was overcome in part by the development of semi-micro methods, greatly decreasing the amount of material needed for analysis. The procedure finally used determines the amide fraction of nitrogen, basic amino, basic non-amino, non-basic amino, and non-basic non-amino ("other") nitrogen in duplicate on as little as 50 cc. of the plant sap (40–50 mgm. N). The general scheme of analysis with details of the methods is presented by Orcutt and Wilson (9).

In the plants receiving combined nitrogen small quantities of NO_3^- were often found in the tissue, particularly after recent application of NH_4NO_3 to the substrate. All data reported here have been corrected for the nitrate-nitrogen present and represent, therefore, the nitrogen that has entered actively into the metabolism of the plant. Ammonia nitrogen was determined with the amide fraction, as previous experiments had shown ammonia to be relatively low and virtually constant, representing about 2 per cent of the total soluble nitrogen.

<div align="center">DATA</div>

Two experiments, considered typical of the several completed, are reported in this paper. The work has been in progress for 3 years, but analyses in three earlier experiments were made with ordinary macro-methods, and the data obtained were not considered so reliable as those reported here. The results, however, were essentially concordant and confirmed the conclusions reached. Details of the experiments follow:

Experiment 1.

    Soluble Nitrogen Constituents

        Leaves, stems, roots—per cent of soluble nitrogen, figure 1

        Leaves, stems, roots—mgm. N per plant, figure 4

        Nodules, figure 3

        Planted May 17, 1935. First harvest was made at 47 days, at which time fixation had been apparent for over a week.

Experiment 2.

    Soluble Nitrogen Constituents

        Leaves, stems, roots—per cent of soluble nitrogen, figure 2

        Leaves, stems, roots—mgm. N per plant, figure 5

        Nodules, figure 3

    Planted June 17, 1935. First harvest in inoculated series (25 days) was made before fixation had begun. The second harvest of the inoculated series (31 days) corresponds to the first harvest of the combined nitrogen series; inoculated plants at second harvest showed first evidence of nitrogen fixation. The last harvest (60 days) was made just at the onset of maturation (flowering).

The data on the uptake of total nitrogen discussed in a previous paper (12) showed that, in general, the rate of nitrogen fixation in the inoculated plant is comparable with the rate of assimilation in the series receiving large quantities of combined nitrogen.

On the basis of the soluble nitrogenous components of roots, stems, and leaves given in figures 1, 2, 4, and 5, the following general statements may be made:

Amide- and ammonia-nitrogen is, in general, lower in the series receiving lower levels of combined nitrogen in accord with its function as reserve and translocatory form (6). The leaves of the plants in both experiments contain less amide than either stems or roots, particularly in the older plants. No difference between the inoculated plants and those receiving combined nitrogen is apparent.

Basic amino nitrogen occurs fairly regularly in the plants, independent of the source of nitrogen. In experiment 2 it is remarkably low in all tissues.

Basic non-amino nitrogen is somewhat higher in the combined nitrogen series of experiment 1 and is generally higher in the leaves of all series than in other tissues of the plant. In experiment 2 it seems to be absent from the tissues of the inoculated plants before fixation begins (31 days).

Non-basic amino nitrogen is remarkably constant in the tissues of all series. With the exception of the inoculated plants of experiment 1, this fraction comprises 50–60 per cent of the soluble nitrogen. In the inoculated plants of experiment 1 it is somewhat lower, its place being taken by "other" nitrogen.

Other nitrogen (non-basic non-amino) is particularly high in the inoculated plants of experiment 1. In general it seems to increase as the plant grows older.

The analyses of the soluble portion of the nodular tissue, given in figure 3, show a rather constant composition on the whole. The outstanding observation is the marked increase in the basic non-amino nitrogen fraction concurrent with the beginning of nitrogen fixation in both experiments (38–48 days). Also it is noted that in the nodules, non-basic amino nitrogen comprises approximately only 30 per cent of the soluble nitrogen as compared with 50–60 per cent in the other tissues of the plant. "Other" nitrogen likewise is high in both experiments but varies considerably.

DISCUSSION

Do the experiments indicate any essential differences in the fractions of soluble nitrogen found in plants fixing elemental nitrogen and in those receiving combined forms? In general the composition of the plants with

respect to these fractions is fairly constant, irrespective of the source of nitrogen. Probably the most noteworthy observation to be made from the data is the remarkable stability of the soluble nitrogenous constituents, especially in the leaves. It would be expected, however, that the tissues involved in
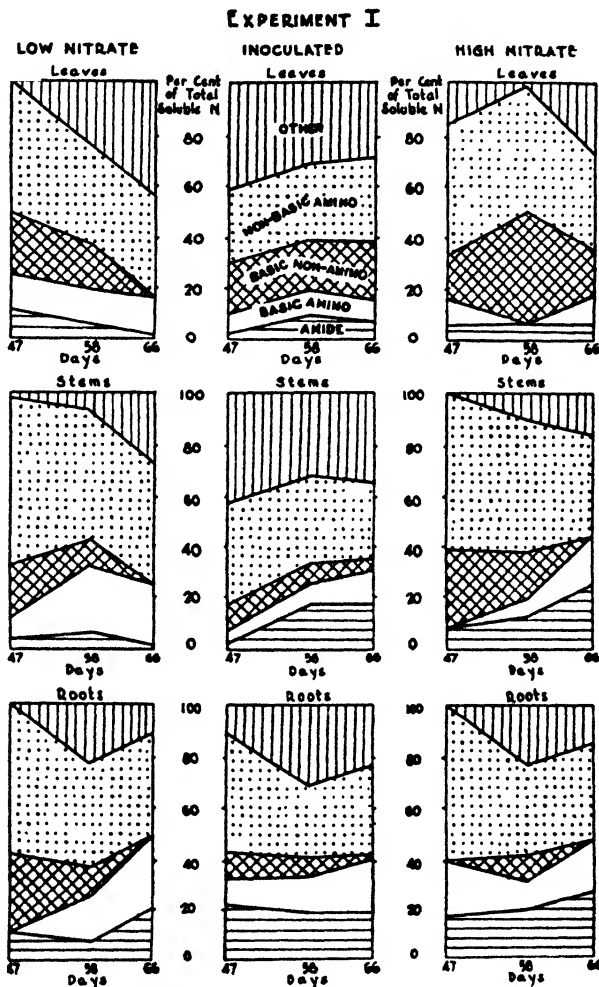
FIG. 1. SOLUBLE NITROGEN FRACTIONS IN ROOTS, STEMS, AND LEAVES OF EXPERIMENT 1 EXPRESSED IN PER CENT OF TOTAL SOLUBLE NITROGEN

(Legend given for *Inoculated Leaves* applies to all graphs)

transportation (roots and stems) from the centers of fixation to the leaves would be more likely to reveal differences arising from the use of the free element. It is of interest to examine the data to determine whether they yield any support to current hypotheses of the mechanism of symbiotic nitrogen fixation.

The ammonia hypothesis

Winogradsky (18) has proposed ammonia as the initial product of fixation, on the basis of the observation that detached nodules liberate NH_3 even in the presence of antiseptics or after drying at 40°C. In his studies no evidence was presented that free nitrogen was actually assimilated; in view of the
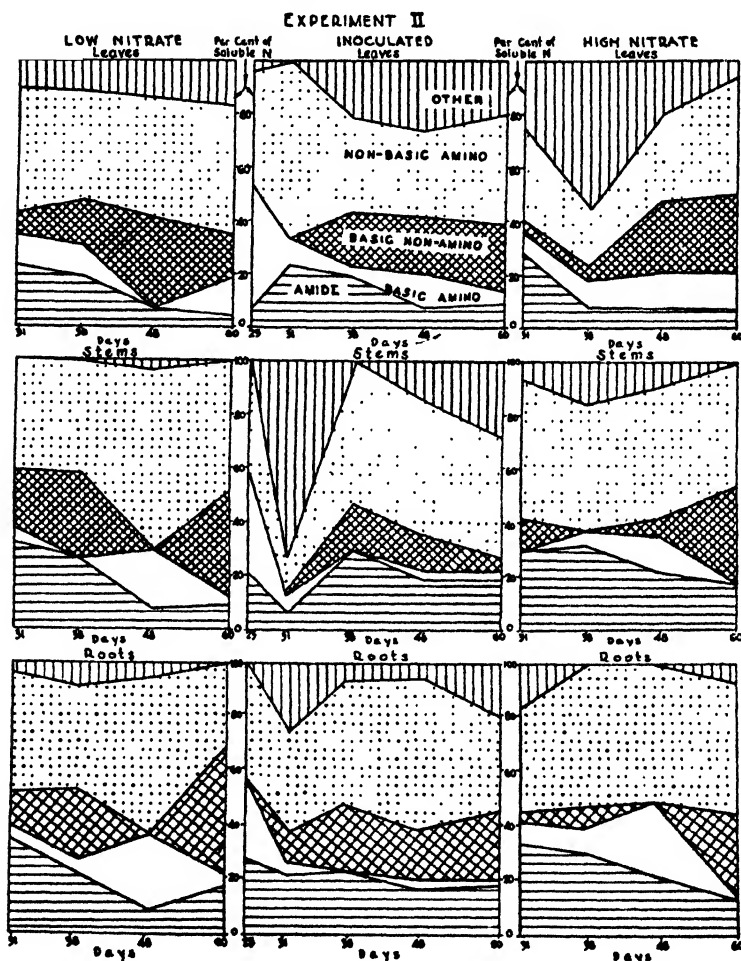
FIG. 2. SOLUBLE NITROGEN FRACTIONS IN ROOTS, STEMS, AND LEAVES OF EXPERIMENT 2 EXPRESSED IN PER CENT OF TOTAL SOLUBLE NITROGEN

(Legend given for *Inoculated Leaves* applies to all graphs)

negative results of other workers (4, 17), it is doubtful whether fixation took place in the detached nodules. The ammonia undoubtedly originated from deaminization of the free amino acids present in the nodules. Burk and Horner (3) have criticized a similar claim of Winogradsky with respect to the finding of NH_3 in cultures of Azotobacter, and their criticisms appear to be

equally valid for the conclusions based on liberation of ammonia from excised nodules. As has been noted, NH₃-nitrogen is low in all the tissues of the soybean. Even in plants supplied with NH_4NO_3 the level of NH_3 was found to be rather low when the combined nitrogen was added no more rapidly than free nitrogen was being fixed. This finding suggests that even though nitrogen
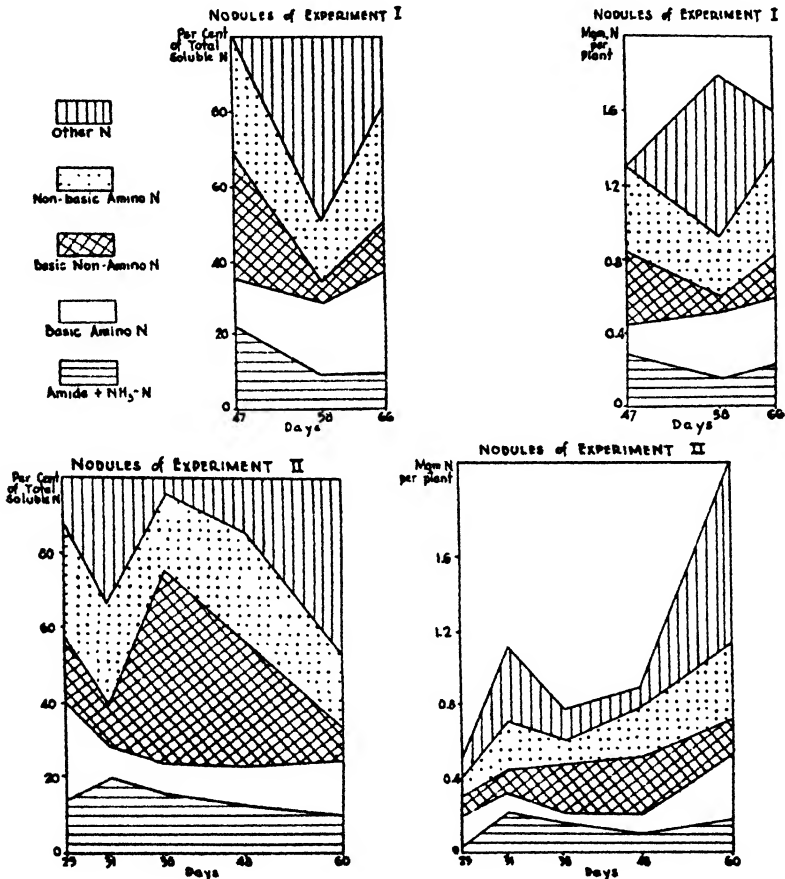
FIG 3. SOLUBLE NITROGEN FRACTIONS IN THE NODULES OF EXPERIMENTS 1 AND 2 EXPRESSED BOTH IN PER CENT OF TOTAL SOLUBLE NITROGEN AND IN MILLIGRAMS PER PLANT

were fixed in this form it would not accumulate; hence failure to detect it may not be significant.

The hydroxylamine hypothesis

Virtanen and collaborators (14, 15) have shown that leguminous plants, at least under certain conditions, excrete aspartic acid and beta-alanine into the sand substrate in which the plant is grown. On the assumption that

these amino acids represent compounds excreted from the bacteria, Virtanen and Laine (14) suggest that the initial product of fixation is NH_2OH, which is then combined with oxalacetic acid to form the oxime. This compound is then reduced to aspartic acid that is excreted to the plant or substrate. The
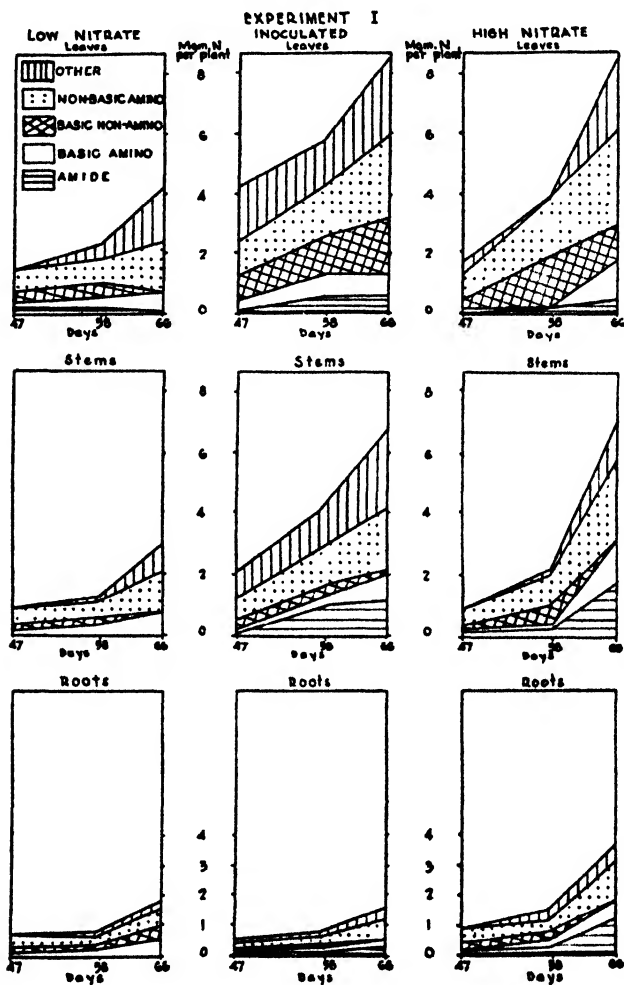
FIG. 4. SOLUBLE NITROGEN FRACTIONS IN ROOTS, STEMS, AND LEAVES OF EXPERIMENT 1 EXPRESSED IN MILLIGRAMS PER PLANT

(Legend given for *Low Nitrate Leaves* applies to all graphs)

root nodule bacteria in the substrate split off CO_2 from part of the excreted aspartic acid to form beta-alanine. Isolation of small quantities of oximes from the sand substrate is offered as confirmatory evidence for the hypothesis. Although of interest, the basic assumption that the aspartic acid *must* be intermediate in the fixation reaction is as yet unproved. The fact that this

amino acid is the only nitrogen compound excreted from the nodule may signify only that the cells of the nodule are, under certain conditions, permeable to this particular acid. It should be noted that the leaves of rye grass may excrete glutamine when the plant is supplied with ammonium salts (5);
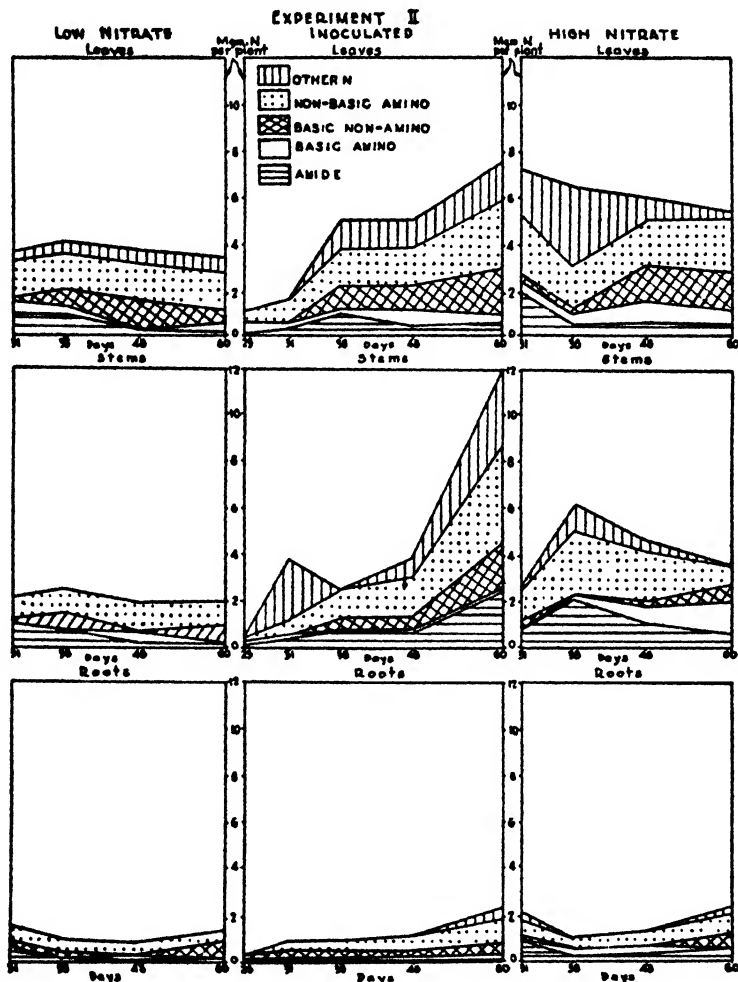
FIG. 5. SOLUBLE NITROGEN FRACTIONS IN ROOTS, STEMS, AND LEAVES OF EXPERIMENT 2
EXPRESSED IN MILLIGRAMS PER PLANT

(Legend given for *Inoculated Leaves* applies to all graphs)

hence excretion of a nitrogenous compound is not necessarily significant for the fixation process.

In the experiments reported in this paper no evidence was uncovered that supported the hydroxylamine hypothesis. Non-basic amino acids, which would include aspartic acid, were actually found to be lower in the nodules

than in other tissues of the plant.   There was no accumulation of this fraction in the nodule, and no increase in the relative quantity was found when fixation began.   Since the fraction occurred in equally large quantities in plants receiving combined nitrogen as in those fixing free nitrogen, the finding of high percentages of non-basic amino nitrogen in the transporting tissues of the inoculated plants cannot be taken as necessarily significant for the process of nitrogen fixation.   More detailed analysis of the nodules and other tissues, in order to determine whether aspartic acid comprises more of the non-basic amino fraction in plants fixing free nitrogen than in those given combined forms, would be of value.

The amide hypothesis

A second source of the excreted aspartic acid might be asparagine.   Burk and Horner (2) state that the evidence dealing with the analogous problem of non-symbiotic nitrogen fixation by Azotobacter favors the view that an amide is intermediate in the fixation reaction.   A similar finding for the symbiotic process might be expected.   There is little indication, however, in the results of these experiments that amides have a specific function in nitrogen fixation in the soybean.   The level of amide nitrogen in the roots, stems, and leaves appears to be independent of whether the source of nitrogen is free or combined. In the nodules 10–20 per cent of the total soluble nitrogen is present as amide-N, but the level does not increase with the onset of fixation.

As these data do not appear to offer positive support for any of the current hypotheses, it is of interest to consider some of the other nitrogen fractions not involved in these hypotheses.

Other nitrogen

Other nitrogen includes non-basic non-amino nitrogen and contains proline as well as other unknown compounds.   Reference has been made to the occurrence of a high percentage of other nitrogen in the inoculated plant, particularly in experiment 1.   In experiment 2 it was noted that other nitrogen fulfilled a much less significant function and is reasonably comparable to that found in the plants receiving combined nitrogen.   It will be noted further that other nitrogen increases slightly at maturation (which may be the explanation of its appearance in experiment 1), in agreement with the work of Thomas (10), who suggested that other nitrogen represents degraded or catabolic forms.   This type of nitrogen is conspicuous when assimilation is decreased, whatever the cause.   Furthermore if other nitrogen includes a simple fixation product being transported through the plant, one would expect to find less of it in the root than in the nodule, and still less in the stem and leaf, its place being taken by other transformation products.   Even in experiment 1, however, the content of other nitrogen, although very high in the nodule, is very low in the root, higher in the stem, and still higher in the leaf.   The implication of other nitrogen in the symbiotic fixation process, therefore, will

have to be based on grounds other than the relatively high content of such nitrogen in the inoculated plants of experiment 1.

Basic non-amino nitrogen

It was observed that the inoculated plants apparently contained little basic non-amino nitrogen before fixation began (experiment 2, fig. 2). Indeed in the first two harvests amino nitrogen (Van Slyke) accounted for the total nitrogen in the basic fraction (phosphotungstic acid precipitate); this, however, doubtlessly arises from errors in the methods available when applied to exceedingly small quantities of material (13). The important point is that the basic fractions, and especially the non-amino portion, increase rapidly with fixation. After these plants began to derive nitrogen from the fixation process, and before maturation began, *the proportion of non-amino to amino nitrogen in the basic fraction was slightly but consistently higher in the inoculated plant*, particularly in the nodules, roots, and stems.

In figure 3 it is apparent that after fixation had started and during the vegetative growing period before maturation, the ratio of basic non-amino to basic amino nitrogen in the nodule was exceedingly high compared to the normal distribution in other parts of the plant; e.g., a greatly increased amount of basic non-amino nitrogen appeared in the nodules during this period of rapid fixation (approximately 38–48 days in these two experiments). It will be noticed, as well, that basic amino nitrogen remained reasonably constant (both as percentage of soluble nitrogen and as milligrams N per plant). At approximately 60 days, at the time of flowering, this excessive amount of basic non-amino nitrogen disappeared and was replaced by a higher proportion of other nitrogen in the nodules of the plants in both experiments.

These results suggest that nitrogen compounds present in the basic non-amino fraction may be significant for the symbiotic nitrogen fixation process either as intermediates or as the form in which the nitrogen is transferred to the host. In the young nodule the fixation rate is so rapid that a high proportion of this fraction accumulates before condensation or translocation can occur. This view is essentially consistent with the data from all of our experiments, both those presented in this paper and those in the experiments not reported. It is not too apparent in experiment 1, but it should be remembered that these were older plants. The composition of plants of experiment 1 is very similar to that of plants of experiment 2 after 48 days.

It is emphasized that the conclusions drawn from these experiments can be regarded only as suggestive leads. Because of the inherent difficulties in the interpretation of data which include a summation of the reactions concerned with other processes of the plant as well as with nitrogen fixation, little more than suggestions may be expected. The fact that the results support none of the current hypotheses means only that no new evidence has been found in favor of these. The negative results in this respect may be merely an indication that changes in the specific compounds involved in the fixation

reaction are masked by the presence of other metabolic reactions in which these same or similar compounds are concerned. On the other hand it must be remembered that the evidence in support of the NH_3, NH_2OH, or the amide hypothesis (with respect to symbiotic nitrogen fixation) is very meager.

The positive conclusions from these experiments, viz., that the nitrogen represented by the basic non-amino fraction (probably composed of histidine and arginine-like compounds) may be related to the symbiotic nitrogen fixation process, are based upon the same type of evidence that has been presented for the other hypothesis. Such evidence reduces essentially to this: Results obtained from a *particular* technic suggest a *tentative* hypothesis. In this particular case metabolism studies on the plant indicate basic non-amino nitrogen, and the value of the suggestion is strictly limited to the validity of the method used to acquire the information. With the present methods of study it is improbable that definite proof of the occurrence of an intermediate in the fixation process, supported by sufficient evidence to meet such criteria as have been proposed by Burk (1) as necessary for acceptance of a proposed intermediate, will be obtained. For this reason, then, it seems that the best approach to the problem of intermediates in the symbiotic nitrogen fixation process at this time is to obtain as many suggestions as possible with present methods. As different technics are developed and become more refined, it should be possible to examine critically the various proposals and determine which is correct.

SUMMARY

Experiments were made in which the soluble nitrogen fractions of soybean plants fixing atmospheric nitrogen were compared with those of plants supplied with combined nitrogen. The purpose of the study was to determine whether any particular fraction was specifically associated with the fixation process.

An examination of the data shows no supporting evidence for the existence of the previously suggested intermediates in the fixation (or transport) of elemental nitrogen in leguminous plants, viz., NH_3, NH_2OH, and amide. It is pointed out that the methods used may not allow a compound significantly correlated with the fixation process to be distinguished, because of concomitant reactions involving this compound or similar compounds concerned with other aspects of the metabolism of the plant. Hence negative findings do not constitute disproof of any particular hypothesis.

The only fraction that appears to offer possible significance for the fixation process on the basis of these experiments is that group of nitrogen compounds contained in the basic non-amino fraction.

REFERENCES

(1) BURK, D. Criteria of chemical mechanisms in nitrogen fixation by living forms· *Trans. Second Internatl. Cong. Microbiol.* (London). 1936. (In press).

(2) BURK, D., AND HORNER, C. K. 1935 The specific catalytic rôle of molybdenum and vanadium in nitrogen fixation and amide utilization by Azotobacter. *Trans. Third Internatl. Cong. Soil. Sci.* 1: 152–155.

(3) BURK, D., AND HORNER, C. K. 1936 The origin and significance of ammonia formed by Azotobacter. *Soil Sci.* 41: 31–122.

(4) GALESTIN, C. J. A. 1933 Is elementary nitrogen absorbed by root nodules in the assimilation of nitrogen of air by the legumes? *Chem. Weekbl.* 30: 207–209.

(5) GREENHILL, A. W., and CHIBNALL, A. C. 1934 Exudation of glutamine from perennial rye grass. *Biochem. Jour.* 28: 1422–1427.

(6) MURNEEK, A. E. 1935 Physiological rôle of asparagine and related substances in the nitrogen metabolism of plants. *Plant Physiol.* 10: 447–464.

(7) NIGHTINGALE, G. T. 1937 The nitrogen nutrition of green plants. *Bot. Rev.* 3: 85–174.

(8) ORCUTT, F. S., AND FRED, E. B. 1935 Light intensity as an inhibiting factor in the fixation of atmospheric nitrogen by Manchu soybeans. *Jour. Amer. Soc. Agron.* 27: 550–558.

(9) ORCUTT, F. S., AND WILSON, P. W. 1936 Biochemical methods for the study of nitrogen metabolism in plants. *Plant Physiol.* 11: 713–729.

(10) THOMAS, W. 1927 Nitrogen metabolism of *Pyrus melus*. *Plant Physiol.* 2: 109–137.

(11) UMBREIT, W W., AND BOND, V. S. 1936 Analysis of plant tissue. *Indus. and Engin. Chem.* 8: 276–278.

(12) UMBREIT, W. W., AND FRED, E. B. 1936 The comparative efficiency of free and combined nitrogen for the nutrition of the soybean. *Jour. Amer. Soc. Agron.* 28: 548–555.

(13) UMBREIT, W. W., AND WILSON, P. W. 1936 Determination of basic nitrogen. A semi-micro method applicable to plant tissues. *Indus. and Engin. Chem., Anal Ed.* 8: 361–362.

(14) VIRTANEN, A. I, AND LAINE, T. 1936 Fixation of nitrogen in the leguminous root nodules. *Suomen Kemistilehti B* 9: 12.

(15) VIRTANEN, A. I., AND LAINE, T. 1937 Formation of β-alanine from aspartic acid through the legume bacteria. *Suomen Kemistilehti B* 10: 2.

(16) WEBSTER, J. E. 1928 Nitrogen metabolism in the soybean. *Plant Physiol.* 3: 31–45.

(17) WILSON, P. W., HOPKINS, E. W., AND FRED, E. B. 1932 The biochemistry of nitrogen fixation by Leguminosae. I. Nitrogen fixation studies of Rhizobia apart from the host plant. *Arch. Mikrobiol.* 3: 322–340.

(18) WINOGRADSKY, S., AND WINOGRADSKY, H. 1936 Recherches sur les bacteries radicicoles des légumineuses. *Ann. Inst. Pasteur* 56: 221–250.

EFFECT OF FERTILIZERS ON SOME NITROGENOUS AND OTHER CONSTITUENTS OF THE COTTON PLANT AS SEPARATED BY ELECTRODIALYSIS AT DIFFERENT STAGES OF GROWTH[1]

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INTRODUCTION

The study reported constitutes a phase of the program of the Division of Soil Fertility Investigations, Bureau of Plant Industry, dealing with the use of fertilizers, crop residues, and cultural practices as related to the fertility of the soils of the Blackland prairie region of Texas. Previous investigations reported by this division (10) indicate the need for an index of the effect of the fertility of the soil, both inherent and induced, upon the composition of the cotton plant, in order to further an understanding of the incidence of root rot, which is prevalent on many soils of this region. As a part of the general study, investigations have been conducted and reported by Ergle (5) on the periodic changes of some of the carbohydrate components of field-grown cotton plants throughout the growing season. Correlated investigations have been made of the nitrogenous constituents. The present report deals with the experimental technic developed for separating the nitrogen fractions and certain inorganic ions by electrodialysis of the plant tissues and presents preliminary data typical of the results obtained by the procedure.

Most of the studies of the composition of the cotton plant have dealt with the mineral components, and large differences (2, 8, 11) have been obtained for the different plant parts. The nitrogen content varied with plant development (20, 21). Although variable results have been obtained, a general conclusion is that a relative abundance of soil nitrogen induces a high content in the plant; the greatest increase takes place soon after the application of a soluble-nitrogen fertilizer to the soil (1, 2). The soluble nitrogenous fractions of the cotton plant have been studied by Davidson, Clark, and Shive (4). Holley and his associates (9) have reported on the utilization of ammonia and nitrate nitrogen by cotton and the effect of these forms on the fruiting and on certain organic components of the plant.

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[1] Plans for these studies were developed by the authors and P. R. Dawson, then in charge of the soil fertility-cotton root-rot investigations at Austin, Texas, and were carried out under the direction of J. E. Adams, now in charge at Austin, Texas. The work was under the general supervision of J. J. Skinner, in charge of cotton soils and fertilizer investigations.

[2] Now agronomist with the North Carolina Agricultural Experiment Station.

METHODS

Sampling

The cotton plant samples for electrodialysis were collected from field plots, as described for the carbohydrate studies reported elsewhere (5). The same details of selection and precautions to insure representative samples were observed in this case. The plants were separated into top and root portions, weighed, and ground in a food chopper.

Preservation of samples

Aliquots for analysis were preserved by processing with steam under 15 pounds pressure in sealed tin cans. The samples in the sealed cans were then transferred to a sub-freezing temperature until analyzed. This method of preservation was adopted after considerable preliminary experimentation and gave results that were comparable with those obtained with fresh samples. In investigations of this character, it is imperative that the plant materials be preserved by a method that will reduce as far as possible the effects of chemical and enzymatic changes in the nitrogen constituents and will permit comparable results from large numbers of samples collected on different dates and at different locations. The various methods of preservation of plant samples have been evaluated by Tottingham and his associates (17); apparently no method is without theoretical objections. Since, in the electrodialysis procedure used, the sample is subjected to room temperature for 23 hours, a preliminary heat treatment of the ground plant material for complete inactivation of enzymes is essential. This is accomplished by the procedure adopted.

The electrodialysis apparatus and technic

Electrodialysis was adopted for the extraction and partial separation of the ionic fractions of the cotton plant, as this procedure seemed to avoid many of the difficulties pointed out by Vickery (19) in the conventional methods of studying the nitrogenous constituents of green plants. In this way the extract was separated into two parts containing the positive and negative ions, and most of the pigment and other interfering substances were eliminated. Tiedjens (16) applied the electrodialysis method to the study of the nitrogenous fractions of plant material, and Paden (14) used it in the extraction of cations from cotton tissue.

The apparatus used is shown in plate 1. It is a 3-compartment Mattson-type cell, as modified and developed by the Bureau of Chemistry and Soils. The dimensions of the electrode compartments were approximately 150 x 90 x 19 mm., with capacities of about 240 cc. each. The central chamber was of the same dimensions as the electrode compartments when small samples (60 gm., root material) were dialyzed; for larger samples (120 gm., tops), the capacity was increased by inserting an additional U-shape rubber section.

The electrode distances were $1\frac{5}{8}$ and $1\frac{11}{16}$ inches, respectively.   Cellophane membranes (0.0015 in.) were used, with plantinum gauze for the anode and copper gauze for the cathode.   In the routine work two of these cells were operated in parallel.

The procedure developed for carrying out the electrodialysis was as follows: After transfer of the sample to the central chamber, with rinsing, 200 cc. of water was added to each of the electrode compartments, and the cooling coils and electrodes were inserted.   Sufficient water was then added to the central chamber to raise the general level of the liquid above the top of the electrodes. Electrodialysis was effected with a potential difference of 100–110 volts, using a 100-watt lamp in series with each cell as a protective resistance.   The average initial amperage for each cell was approximately 0.8.   This dropped to approximately 0.2 ampere at the end of the dialysis with the smaller samples, but with the larger samples the drop was not so great.   A few drops of amyl alcohol materially reduced foaming in the electrode compartments during the later stages of dialysis.

The electrode compartments were drained at definite intervals of increasing length, and water was added to re-establish the level in the compartments. Drainage was effected a total of 5 times within the 23-hour period for the small, and 7 times for the large, samples.   The solution withdrawn from the cathode chamber was acidified with acetic acid (1:9) during the earlier part of the investigation; later, the titer was determined by adding an excess of sulfuric acid and back-titrating.   Toluene was used as a preservative in all cases.

After the last drainage, the solid organic material which had collected in the cathode chamber and the film of calcium carbonate adhering at the solution level were removed with acid, and the rinsings were combined with the dialysate.   The contents of the central compartment were quantitatively removed and dried; they are hereafter referred to as the "residue after dialysis."

Preparation of the dialysates

The contents of the flask containing the solution and washings from the cathode chamber were filtered through a Büchner funnel.   The filtrate, which was almost colorless, was made up to 2,000 cc. when the sample was 120 gm., and to 1,250 cc. when it was 60 gm.   The anode portion, always yellow in color, was made up to the same volume as the cathode solution without filtration.

Ammonia nitrogen

The quantity of ammonia present was determined by an adaptation of Folin's aeration method (7).   Fifteen grams of anhydrous sodium carbonate was added to a 100 cc. aliquot from the cathode chamber, and the ammonia collected in an absorption tube containing 5 cc. of 0.05 N sulfuric acid.   After 20–22 hours of rapid aeration the absorption tube was disconnected, and the excess acid titrated with methyl red as an indicator.

Amide nitrogen

Fifty cubic centimeter aliquots were removed from both the cathode and anode solutions, as both contained amide nitrogen. The combined sample was made approximately 5 per cent acid with concentrated sulfuric acid, and the amides were hydrolyzed by heating in a boiling water bath for 2 hours After cooling, concentrated sodium hydroxide was added below the surface of the liquid until the solution was only slightly acid to phenolphthalein. Following the addition of 15 gm. of sodium carbonate, the total ammonia was determined by aeration. The amide nitrogen was calculated after a correction was made for the quantity of ammonia originally present in the solution.

Basic nitrogen

A 200 cc. portion of the cathode solution was made neutral to methyl red, evaporated on a steam bath to slightly less than 50 cc., allowed to cool, and made up to 50 cc. After the solution had been made 5 per cent acid with concentrated sulfuric acid, the basic nitrogen was precipitated by means of 10 cc. of phosphotungstic acid solution, according to the method of Osborne and Harris (13). Whereas the precipitate in the solution from the tops of the plants was always somewhat flocculent and readily filtered, that from the roots was more granular in nature and much finer, being retained by the filter paper with difficulty. The nitrogen in the precipitate was determined by a Kjeldahl analysis.

Total cathode nitrogen

The nitrogen in 50 cc. of the cathode dialysate was determined by the Kjeldahl method.

Total anode nitrogen

An aliquot in a Kjeldahl flask was made barely alkaline with sodium hydroxide, the water evaporated on a steam bath, and the nitrogen determined by the salicylic acid-sodium thiosulfate method (3, p. 21).

Nitrate nitrogen

An aliquot of the anolyte (5–20 cc.) was treated with silver sulfate, to precipitate the chlorides, followed by a small amount of calcium oxide. Occasionally some pigment remained after this treatment and was removed with alumina cream. The nitrate nitrogen was then determined colorimetrically (3, p. 405).

Calcium

The calcium in the 50 cc. aliquot of the cathode solution was determined by precipitation as the oxalate and titration with 0.1 N potassium permanganate, according to the second procedure given in the "Official Methods" (3, p. 104).

Phosphate

The concentration of phosphate in the anolyte was determined by the colorimetric method described by Truog and Meyer (18). Although, in general, a 10 cc. aliquot from the anode portion was diluted to 250 cc. and 50 cc. of this solution taken for the analysis, greater dilutions were frequently necessary.

Residue after dialysis

After the residue from the central chamber had been dried to constant weight at 100°, it was analyzed for nitrogen, using the Kjeldahl procedure.

Titration of the catholyte

An aliquot (50 cc.) of the acidified catholyte was back-titrated to the methyl red endpoint with 0.1 N sodium hydroxide. The number of cubic centimeters of normal acid required to neutralize the base in 100 gm. of green material was then calculated.

Titration of the anolyte

An aliquot (50 cc.) was titrated to the phenolphthalein endpoint with 0.1 N sodium hydroxide. The result was expressed as the number of cubic centimeters of normal base required to neutralize 100 gm. of green material.

RATE OF ELECTRODIALYSIS

Samples of plant tops taken from plats receiving widely different treatments were dialyzed as outlined, but for an additional 5-hour period (total of 28 hours). The individual drainings were analyzed for total anode and cathode nitrogen, ammonia, nitrate, phosphate, calcium, and the titration value of the cathode and anode solutions. The results are shown graphically in figure 1.

Practically all of the ions were removed in the 23-hour period; the rate of removal reached a constant value, so the data are comparable. Nitrate nitrogen was removed completely in 5 hours, whereas the removal of the major portion of the ammonia nitrogen required 15 hours. This latter rate differs markedly from that recorded by Tiedjens (16) for different plant material, where a complete migration of the ammonium ion was effected in 15 minutes. The inorganic ions were removed more rapidly than the organic.

Periodic tests of the solution from the sample compartment showed increases in acidity. These changes are reflected in the "base in cathode" and "acid in anode" curves, which show that the removal of cations is more rapid than that of the anions. The milliequivalents of cations removed is also greater. Reiner (15) and Ettisch and de Loureiro (6) found that the final pH of the central chamber approached the isoelectric point of the non-diffusible ampholyte present; inasmuch as the isoelectric point of plant proteins is about pH 3.3 to 4.5 (12), it would seem that the removal of more cations than anions would be required to bring about a change of the pH.

The amount of dialyzable material in the roots is less than in the tops; this fact is also indicated by the rapid decrease in the amount of the current carried.
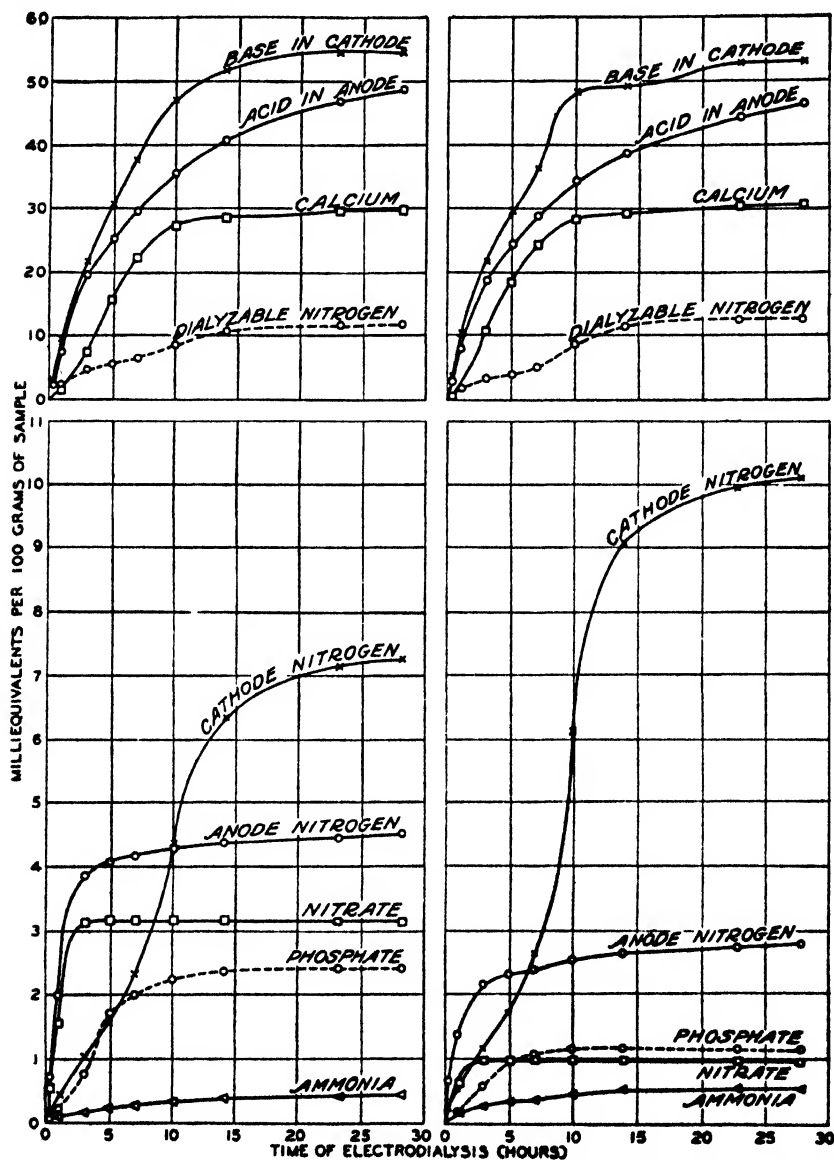
FIG. 1. TIME REQUIRED FOR ELECTRODIALYSIS OF IONIZABLE COMPONENTS OF THE COTTON PLANT: LEFT, SAMPLE 1; RIGHT, SAMPLE 2

A yellow pigment always appeared in the anode chamber, principally during the last 8 to 10 hours of dialysis. A brown to black sludge, alkaline in

reaction and soluble in acid, accumulated in the cathode compartment during the last three drainage periods.   Less of this precipitate occurred during the dialysis of old plants than of young ones.

ANALYTICAL DATA FROM FIELD SAMPLES AND TREND OF RESULTS

Data were obtained from the application of the procedure developed and described above to cotton plants sampled at regular and frequent intervals during the growing season.[3]

Field A was on a Wilson clay loam in Travis County, near Elgin, Texas.
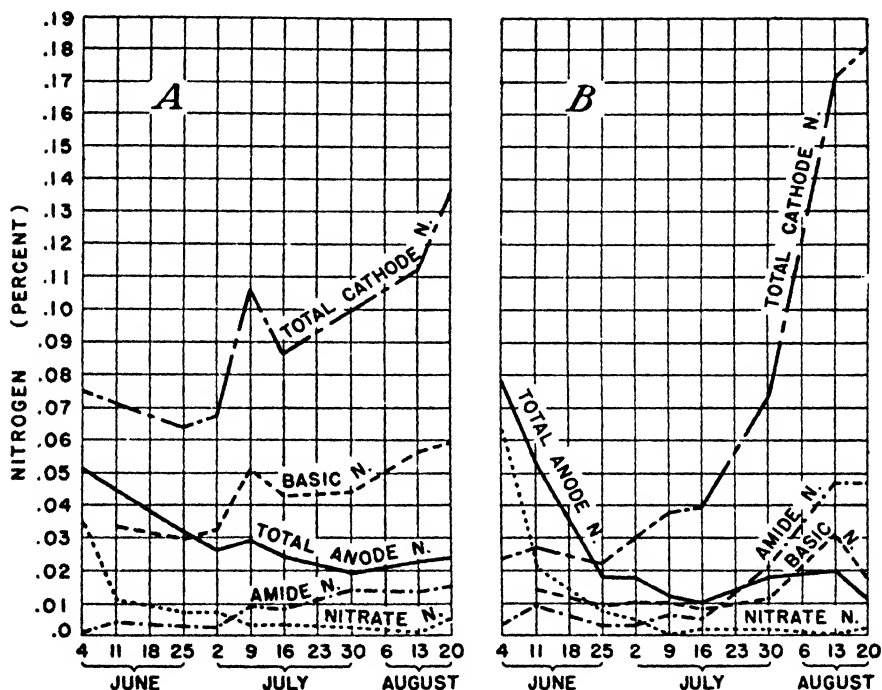
FIG. 2. NITROGEN CONSTITUENTS OF COTTON PLANTS GROWN ON UNFERTILIZED WILSON CLAY LOAM AT DIFFERENT STAGES OF DEVELOPMENT AS DETERMINED BY ELECTRODIALYSIS: *A*, PLANT TOPS; *B*, PLANT ROOTS

Four fertilizer treatments,[4] namely, 0-15-0, 15-0-0, 3-9-3, and 9-3-3, and an unfertilized check plat replicated 5 times were used.   The nitrogen of the fertilizer was derived from equal quantities of sodium nitrate and ammonium sulfate, the phosphoric acid from superphosphate (18%), and the potash from sulfate of potash.   Choice of comparable plants for samples was based upon

[3] The authors are indebted to H. V. Jordan and J. H. Hunter of the Division of Soil Fertility Investigations for the care of the plats and assistance in the collection of plant samples.

[4] Fertilizer ratios are given in the order of N-P$_2$O$_5$-K$_2$O.

their height, number of nodes, squares, flowers, and bolls, determined on the day of sampling, as described by Ergle (5). Equal numbers of plants from each of the respective replications were composited, from which samples for dialysis were taken. Samples were taken from the time the plants were beginning to set squares until approximate boll maturity.

Field B was located on a non-calcareous phase of Houston black clay at the United States Cotton Breeding Station in Hunt County, near Greenville, Texas. This field received 7,000 pounds of 4-10-0 fertilizer per acre in the fall of 1931 and 1932, and 900 pounds of 9-3-0 in the spring of 1934. In 1935 when cotton plants for analyses were taken, 1,000 pounds of a 9-3-0 fertilizer per acre was applied. Plant samples were taken from this section and also from an area on each side of the plats which received no fertilizer. The sampling of cotton plants for analyses began on August 2, just after boll formation, and continued at weekly intervals for 5 weeks.

In figure 2 are plotted the concentrations of some of the nitrogen fractions in the tops and roots, respectively, of cotton plants grown on unfertilized Wilson clay loam. Beginning with June 4, plant samples were taken at weekly intervals until August 20, but analyses were not made on samples taken on June 18, July 23, and August 6. June 4 is the period in plant development when the plants are beginning to produce squares. July 2 to July 9 represents the period when bolls begin to form; July 23 and 30, when boll formation is at its height; and August 6, 13, 20, when bolls are maturing and opening. This chart shows the trends of these nitrogen fractions in the plant at various stages of development. Inasmuch as the experiment is for a single year and the work is in progress, the analytical data are not given in detail. From the data obtained by the methods developed, the following trends were noted.

The ammonia concentrations in the cotton plants, on both Wilson clay loam and Houston black clay, were relatively low throughout the growing period and practically the same in tops and roots. There was a slightly greater concentration in the plants grown on both soils where fertilizers containing nitrogen were used; however, there was no consistent change in concentration with development of the plant. Nitrate concentration was maintained at a high level in the tops of plants grown with high nitrogen fertilizers through July 2, whereas in plants fertilized with the no, or low, nitrogen mixtures, the concentration decreased abruptly immediately following the June 4 sampling. In the roots, the concentrations in all cases decreased rapidly after this date. The high level of nitrate nitrogen during the early stages of growth appears to be characteristic of cotton plants grown on these soils. Ergle (5) has shown that these plants contain limited quantities of carbohydrates at this time. It would seem that the concentrations of nitrates and carbohydrates bear an inverse relation, for during later stages of growth the concentration of carbohydrates is high and that of nitrates is low.

The concentration of amide nitrogen in both root and top samples was uni-

formly low until July, or when bolls were forming. Following this time, although the roots contained appreciably greater quantities than the tops, the concentration in both parts increased as the season advanced. Phosphate fertilizer depressed amide nitrogen with respect to the check, whereas fertilizers containing nitrogen increased it.

The concentration of basic nitrogen, which is that part precipitated from the catholyte by phosphotungstic acid, was lower in the roots than in the tops of samples from both fields; it increased as the season advanced. The high phosphate fertilizer definitely reduced this form in the tops but exerted a less consistent effect on the concentration in the roots. The high nitrogen ratios increased the concentration in both tops and roots. A decline during the early stage of plant development in the total cathode nitrogen, which includes the ammonia, part of the amide, and all the basic nitrogen of the plants, was more evident in the tops than in the roots. The subsequent increase throughout the remainder of the season, however, was observed to be more rapid in the roots, and the final level was appreciably higher. An increased concentration was obtained in both plant parts from nitrogen fertilizers, the relative effect was in proportion to the amount of nitrogen in the fertilizer. The phosphate fertilizers reduced the concentration below that of the check.

The total anode nitrogen, including nitrates and other unidentified nitrogen forms, was maintained at a generally higher level in the tops by the high nitrogen fertilizers. This high level increased gradually and reached a constant level about July 16. The concentration in the plants grown with high phosphate fertilizer dropped abruptly between June 4 and June 11. The root samples were characterized at the beginning of the study by decidedly higher concentrations than those of the tops but after a rapid decline during the period of square formation were uniformly lower. The high-nitrogen fertilizers produced higher concentrations than the others throughout the entire season. The rapid decreases noted for the early part of the season are largely due to the disappearance of nitrate nitrogen in the plants. The total anode nitrogen in the tops was of about the same magnitude as the total cathode nitrogen in early-season plants from the high nitrogen plats. The initial percentage of anode nitrogen in roots from all treatments was higher than that of cathode nitrogen. In general, the ratio of total cathode to total anode nitrogen is lower in plants from high-nitrogen than from high-phosphate plats.

The residual nitrogen does not show any consistent variation with the development of the plant or appreciable differences due to fertilizer treatments. The concentration of inorganic phosphate was high in plants fertilized with high-phosphate fertilizers during early growth but decreased rapidly from then until June 25, the period of boll formation; the level was fairly constant throughout the rest of the season. The concentration of calcium was considerably greater in the tops than in the roots, showing an upward trend until boll maturity was reached and then remaining constant until the end of the

season.   Calcium concentration in the roots remained fairly uniform through-
out the season.

SUMMARY

Methods for preparation of samples of cotton plant material and procedures
by electrodialysis technic adaptable to plant biochemical studies were de-
veloped and are given in detail.   Interfering substances are largely eliminated
by this method of separation of the nitrogen fractions.   A study of the rate
of electrodialysis indicates that practically all of the ions are removed within
23 hours.

Cotton plants used in this study were grown with and without fertilizers
on Wilson clay loam and Houston black clay, two of the principal soil types
of the Blackland section of Texas.   The roots and tops of the plants were
analyzed periodically during the season by the electrodialysis technic for
nitrogen fractions, inorganic phosphate, and calcium.

The data showing the type of results which may be obtained by the pro-
cedure developed may be summarized as follows.

The nitrogen fractions of the cotton plant reflect soil conditions and seasonal
variations better than the total nitrogen.   Ammonia nitrogen was relatively
low in the plants throughout the growing period; amide nitrogen was also
low during the earlier stages, but increased as the season advanced.   The
roots contained more of the latter fraction than the tops.   The trends for total
cathode nitrogen and for basic nitrogen, its principal constituent, are similar.
The slight decline during the early development of the plant was more notice-
able in the tops, but the subsequent pronounced increase was more rapid in
roots.   The tops contained more basic nitrogen than the roots.   The nitrate
content decreased from a relatively high concentration early in the season to a
relatively low one during boll formation; this low value was subsequently
maintained.   The total anode nitrogen follows closely the trends exhibited by
nitrate nitrogen, which constitutes the more variable portion of this fraction.
Plants grown on soils treated with high nitrogen fertilizers contained a greater
concentration of all the nitrogen fractions than those grown on untreated
soils.   A greater concentration of phosphate was found in the tops than in
the roots.   The phosphoric acid concentration was generally in proportion to
the phosphoric acid content of the fertilizer used.   The concentration of
calcium in the tops increased with plant maturity.   The calcium content of
the plant was not materially affected by fertilizers on these soils.

REFERENCES

(1) ANDERSON, J. T.   1894   Fertilizers required by cotton as determined by the analysis
       of the plant.   Ala. Agr. Exp. Sta. Bul. 57.
(2) ARMSTRONG, G. M., AND ALBERT, W. B.   1931   A study of the cotton plant with
       especial reference to its nitrogen content.   *Jour. Agr. Res.* 42: 689–703.
(3) Association of Official Agricultural Chemists   1930   Official and Tentative Methods of
       Analysis, ed. 3, Washington, D. C.

(4) DAVIDSON, O. W., CLARK, H. E., AND SHIVE, J. W. 1934 Preparation of aqueous extracts of soluble nitrogen from plant tissues. *Plant Physiol.* 9: 817–822.

(5) ERGLE, D. R. 1936 Carbohydrate content of cotton plants at different growth periods and the influence of fertilizers. *Jour. Amer. Soc Agron.* 28: 775–786.

(6) ETTISCH, G., AND DE LOUREIRO, J. A. 1933 Zur Methodik der Elektrodialyse (die Serumelektrodialyse mit der Glykokollmembran). *Biochem. Ztschr.* 266: 422–435.

(7) FOLIN, O. 1902 Eine neue Methode zur Bestimmung des Ammoniaks im Harne und anderen thierischen Flussigkeiten. *Ztschr. Phys. Chem.* 37: 161–176.

(8) FRAPS, G. S. 1919. The chemical composition of the cotton plant. Tex. Agr Exp. Sta. Bul. 247.

(9) HOLLEY, K. T., PICKETT, T. A., AND DULIN, T. G. 1931 A study of ammonia and nitrate nitrogen for cotton: I. Influence on absorption of other elements. Ga. Agr. Exp. Sta. Bul. 169.

(10) JORDAN, H. V., DAWSON, P. R., SKINNER, J. J., AND HUNTER, J. H. 1934 The relation of fertilizers to the control of cotton root rot in Texas. U. S. Dept Agr. Tech. Bul. 426.

(11) MURPHY, H. F. 1936 The nitrogen, phosphorus and calcium content of the cotton plant at pre-blooming to early boll stages of growth. *Jour. Amer. Soc. Agron.* 28: 52-57. •

(12) ONSLOW, M. W. 1931 The Principles of Plant Biochemistry. Part I. Cambridge University Press, Cambridge.

(13) OSBORNE, T. B., AND HARRIS, I. F. 1903 Nitrogen in protein bodies. *Jour. Amer. Chem. Soc.* 25: 323–352.

(14) PADEN, W. R. 1935 Relation of fertilizer treatments to the mineral nutrients in sap and tissue of the cotton plant. Assoc. Southern Agr. Workers. *Proc. 34th, 35th, and 36th Ann. Conv.* 1933, 1934, 1935: 473–474.

(15) REINER, L. 1926 Über einen einfachen Elektrodialysierapparat und über die Grundlagen der Elektrodialyse. *Kolloid Ztschr.* 40: 123–128.

(16) TIEDJENS, V. A. 1934 Factors affecting assimilation of ammonium and nitrate nitrogen, particularly in tomato and apple *Plant Physiol.* 9: 31–57.

(17) TOTTINGHAM, W. E., KERTESZ, Z. I., LOOMIS, W. E., AND PHILLIPS, T. G. 1935 Chemical analysis of plant tissue. *Plant Physiol.* 10: 383–386.

(18) TRUOG, E., AND MEYER, A. H. 1929 Improvements in the Deniges colorimetric method for phosphorus and arsenic. *Indus. and Engin. Chem. Anal. Ed.* 1: 136–139.

(19) VICKERY, H. B. 1930 Some aspects of the chemistry of the green leaf cells. *Sci. Mo.* 31: 408–421.

(20) WHITE, H. C. 1914 The feeding of cotton. *Ga. Agr. Exp. Sta. Bul.* 108: 129–144.

(21) WHITE, H. C. 1915 The feeding of cotton: II. *Ga. Agr. Exp. Sta. Bul.* 114: 257–268.

PLATE 1

ELECTRODIALYSIS EQUIPMENT AND ACCESSORIES

At left is complete assembled cell in operation; at right is dissembled cell with electrodes and rubber parts in foreground and grid-shaped glass cooling coils suspended from water-circulation manifold in center. The source of direct current, a rotary converter, with attached switch panel and meters, is shown in the middle background.
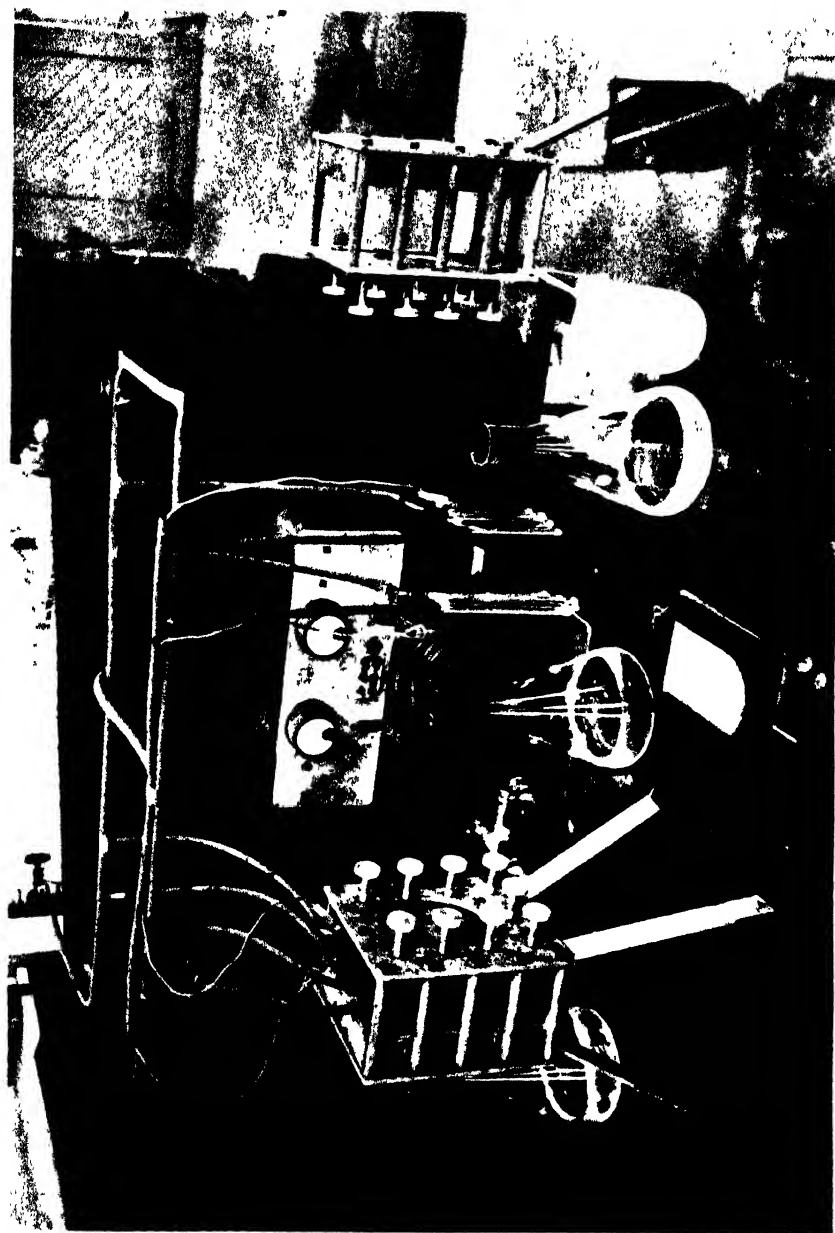
PLATE 1

EMERSON R. COLLINS AND NEIL E. RICLER

NUTRIENT CONTENT AND BASE EXCHANGE PROPERTIES OF ORGANIC LAYERS OF FOREST SOILS IN THE LAKE STATES REGION[1]

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During the last few years large quantities of litter, duff, and humus soil have been used in forest nurseries of the Lake States region for the direct treatment of seedbeds, as compost ingredients, and for the preparation of liquid fertilizers. Although, on the whole, the results of these treatments were very satisfactory (plate 1, fig. 1), a considerable variation has been observed in the effect of organic materials of different origin and morphological features on the growth of seedlings.

In order to obtain general information as to the fertilizing value of various types of organic remains, an experiment was conducted in the greenhouse with a number of humus soils and duffs of known chemical composition. The humus soils and duffs were ground and mixed with quartz sand at the rate of 4 per cent by weight. The mixture was placed in half-gallon glazed jars, in each of which 60 seeds of Norway spruce, *Picea excelsa*, were planted. After 2 years the seedlings were removed, dried in an oven at 60°C., and weighed. The results are presented in table 1. A few results obtained from similar treatments of quartz sand with peats are also included in this table for comparison. Plate 1, figure 2, illustrates the growth effects produced by several types of organic remains.

With a few exceptions the correlation between the growth of the seedlings and the nutrient content of the organic remains is rather close, as shown in figure 1.[3] The low yields produced by mulls are explainable because of their high volume weight. When grain and crumb mulls were applied to the soil in the same volume as the duffs, the productivity of seedlings was about

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[1] Contribution from the soils department, College of Agriculture, University of Wisconsin, in coöperation with Wisconsin Department of Conservation. Publication authorized by the director of the Wisconsin Agricultural Experiment Station.

[2] Assistant professor, chemist, and assistant in soils, respectively. The authors are indebted to Dr. R. J. Muckenhirn for helpful suggestions during the course of this study and in the preparation of the manuscript.

[3] The results obtained with white cedar duff and hemlock litter were disregarded in order not to complicate the diagram. It is obvious that the poor growth of seedlings in these cases was due to the exceptionally high pH value of white cedar duff and an insufficient decomposition of the hemlock litter.

doubled, although it never reached the level produced by the application of hardwood-hemlock duff. Crumb mull produced a comparatively high rate of growth, even though it has a surprisingly low content of available phosphorus. Some of the discrepancies observed may be attributed to the confluence of biological factors, particularly those related to nitrification, ammonification, and nitrogen fixation.

In the spring of 1935 a detailed investigation of the chemical composition of duff and humus layers was undertaken. About 200 representative samples

TABLE 1

Productivity of different types of organic remains as determined by the growth of Norway spruce in the greenhouse

NO.	TYPE OF ORGANIC REMAINS*	REACTION	BASE EX-CHANGE (PER 100 GM)	TOTAL N	AVAIL-ABLE P	AVAIL-ABLE K_2O	RE-PLACE-ABLE Ca	RE-PLACE-ABLE Mg	AV. WEIGHT OF A 2-YEAR-OLD SEED-LING
		pH	m e.	per cent	per cent	per cent	per cent	per cent	mgm.
1	Sphagnum moss peat	3 9	58 3	0 66	0 001	0 017	0 062	0 003	93
2	Carex sedge peat	4.5	129 9	2 16	0 003	0 022	0.332	0 098	205
3	Norway pine duff	5 5	24 0	0 54	0 006	0 054	0 186	0 043	334
4	White pine duff	4 9	48 6	0 70	0 011	0 071	0 306	0 046	445
5	Hemlock duff	4 8	51 9	1 44	0 009	0 049	0 656	0 048	597
6	Hemlock duff	5 5	78 0	1 56	0 008	0 051	0 460	0 062	623
7	Hardwood-hemlock duff	4 9	55 3	1 44	0 012	0 079	0 562	0 031	793
8	Hardwood-hemlock duff	5 5	85 7	1 90	0 016	0 089	1 178	0 176	859
9	Hardwood-hemlock duff	5 5	76 0	1 42	0 015	0 087	1 060	0 191	876
10	Hardwood leaf mull	6 9	66 0	1 10	0 018	0 078	0 940	0 073	675
11	Hardwood crumb mull	5 0	25 8	0.69	0 003	0 046	0 348	0 048	391
12	Hardwood grain mull	6.4	13 2	0 196	0 004	0 036	0 234	0 048	283
13	Hemlock litter	3.9	84 9	0 73	0 003	0 017	0 166	0 010	305
14	White cedar duff	7 8	82 5	1 05	0 012	0 051	1 224	0 261	221

* Types of soil and locations: 1, black spruce swamp, Sawyer County, Wis.; 2, marsh land, Dane County, Wis.; 3, Vilas sand, Goodnow; 4, Vilas sandy loam, Minocqua; 5 and 6, Kennan fine sandy loam, Clam Lake; 7, Vilas sandy loam, Lac du Flambeau; 8 and 9, Kennan fine sandy loam, Clam Lake; 10, Antigo silt loam, Rice Lake; 11, Colby silt loam, Marshfield; 12, Antigo silt loam, St. Croix; 13, Kennan fine sandy loam, Clam Lake (poorly humified duff); 14, calcareous rock outcrops, Sturgeon Bay.

of organic remains, widely distributed in the Lake States region, were collected and analyzed. The determinations and methods of analysis were as follows: pH values were determined by electrometric or colorimetric methods (5); base exchange capacity and replaceable bases, by a modification of the Chapman and Kelley ammonium acetate method (2); total nitrogen, by the standard Kjeldahl procedure (1); nitrates, by the phenol-disulfonic acid method (3); ammonia, by Harper's modification of the colorimetric method (4); and available phosphorus and available potash, by the Truog (6) and Volk-Truog (7) methods, respectively.

The base exchange properties were investigated because they are responsible for the retention of the nutrient bases released in the decomposition of organic matter or derived from fertilizers. The reaction has a direct bearing upon the availability of nutrients and upon the activity of damping-off organisms. The other factors determined are related to the supply of available plant nutrients.
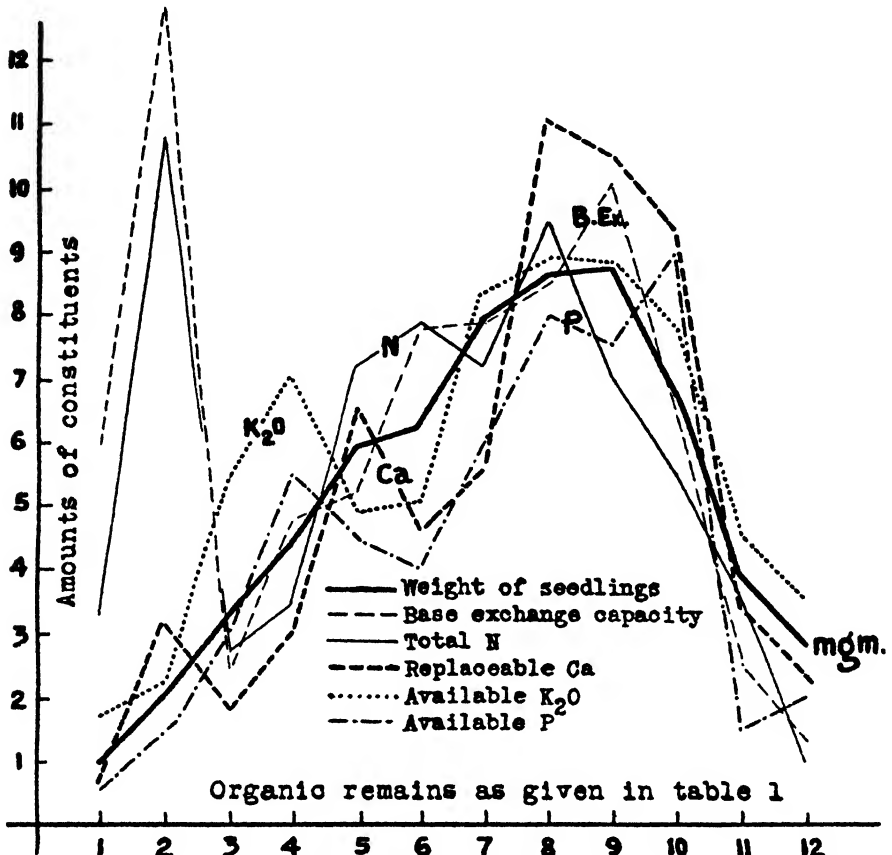
FIG 1. RELATION OF THE BASE EXCHANGE CAPACITY AND OF THE NITROGEN, CALCIUM, POTASSIUM, AND PHOSPHORUS CONTENTS OF DIFFERENT ORGANIC MATERIALS TO THE GROWTH OF NORWAY SPRUCE SEEDLINGS

Each unit of the ordinate represents the following values: 100 mgm. of the dry weight of seedlings; 10 m.e. per 100 gm. of base exchange capacity; 0.2 per cent of total nitrogen; 0.1 per cent of replaceable calcium; 0.01 per cent of available potash; and 0.002 per cent of available Phosphorus.

The investigations were confined to morphological varieties of organic remains occurring on a few widely distributed soil types and under normally developed stands of advanced age. The following varieties were studied: crumb mull humus, formed under stands of sugar maple, basswood, and their

associates on Colby and Vesper silt loams; grain mull humus, from hardwood stands on Antigo, Kennan, Knox, and Miami silt loams; hardwood leaf mull or duff of mull nature, formed on the same soils as the previous variety; jack pine duff, from Plainfield sand; Norway pine duff, from Plainfield and Vilas sands; white pine duff, from Vilas sandy loam; aspen-white birch duff, from Vilas sandy loam; hemlock duff, from Kennan fine sandy loam; hardwood-white spruce-balsam fir duff, predominantly from Colby silt loam; hardwood-hemlock duff, from Kennan loam and Kennan silt loam.[4] With the exception of hardwood mull duff and hemlock duff, all the duffs studied were transitional between leaf mull and true raw humus. The hemlock duff should be considered as a genuine representative of the raw humus type.

A number of analyses were made to determine the variation in the chemical composition of organic remains occurring on the same soil and under the same forest stand. Table 2 gives the results of analyses of seven hardwood-hemlock

TABLE 2

Variations in the chemical composition of duff collected from a hardwood-hemlock stand at different locations

LOCATION	REAC-TION	BASE EXCH (PER 100 GM)	TOTAL N	AV. P	AV. K₂O	REPLACE-ABLE Ca (PER 100 GM.)	REPLACE-ABLE Mg (PER 100 GM)
	pH	m.e.	per cent	p.p.m.	p.p.m.	m.e.	m.e.
Plateau....................	5 3	86.3	1.76	100	668	47.7	10 8
Plateau..........	5.5	85.7	1.90	116	720	58.9	12 6
Hilltop........	5.5	100 0	1 62	150	500	76.0	14 2
Upper slope.....	5 8	76 0	1.14	110	500	49 0	14.5
Lower slope.........	5 3	72.5	1.68	124	626	42 0	10 8
Cove........	5 5	76.0	1 42	100	651	53.0	13.7
Cove...............	5 5	73.2	1 62	110	600	49 3	12.4

duff samples collected on a rolling area of about 5 acres at Clear Lake, near Woodruff, Wisconsin.

The utmost care was exercised in collecting the samples for chemical analysis. In sampling the humus of the earth-mull type, the litter and the earthworms were removed. In sampling duffs, none of the mineral particles from the transitional humus layer were included.

Table 3 presents the mean values of the analyses and the corresponding standard errors. The average values were derived from 7 to 12 determinations. The standard deviation was calculated by dividing the sum of the squared deviations by $n-1$, as this practice gives good results in treating a small number of samples.

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[4] The characteristics of these soil types, as well as the composition of the associated forest stands and ground cover vegetation, have been described in recent soil survey and land utilization publications of the University of Wisconsin and Wisconsin State Departments. For the explanation of terms pertaining to morphology of organic remains see reference (9).

TABLE 3

Fertilizing value of organic remains of upland forest vegetation, as determined by chemical analysis

NO.	TYPE OF ORGANIC REMAINS	REACTION	BASE EXCHANGE (PER 100 GM.)	TOTAL N	NI-TRATE	AM-MONIA	AVAILABLE P	AVAILABLE K₂O	REPLACABLE Ca (PER 100 GM.)	REPLACABLE Mg (PER 100 GM.)
		pH	m.e.	per cent	p.p.m.	p.p.m.	p.p.m.	p.p.m.	m.e.	m.e.
1	Jack pine duff	5 6 ± 0 1	13 3 ± 1 0	0 372 ± 0 034	2 4	9	24 ± 5 7	186 ± 26 7	7 5 ± 0 5	1 8 ± 0 1
2	Norway pine duff	5 4 ± 0 2	29 3 ± 3 6	0 653 ± 0 060		19	38 ± 4 8	287 ± 14 2	12 9 ± 1 7	3 2 ± 0 3
3	White pine duff	5 4 ± 0 2	47 1 ± 3 6	0 976 ± 0 110	T	32	60 ± 7 0	483 ± 32 9	28 2 ± 3 1	5 6 ± 0 9
4	Aspen-birch duff	5.7 ± 0 1	39 3 ± 2 1	0 775 ± 0 160	2 2	30	69 ± 8 7	471 ± 27 0	27 3 ± 1 9	4 8 ± 0 3
5	Hemlock duff	4 8 ± 0 2	73 2 ± 8 6	1 490 ± 0 042		40	57 ± 3 4	432 ± 19 7	29 4 ± 4 7	6 5 ± 1 4
6	Hardwood-hemlock duff	5 4 ± 0 4	72 5 ± 6 2	1 430 ± 0 070	5 4	36	148 ± 9 4	741 ± 29 0	44 1 ± 6 3	8 7 ± 1 9
7	Hardwood-spruce-fir duff	5 5 ± 0 2	65 8 ± 9 3	1 290 ± 0 130	15 0	67	66 ± 2 7	545 ± 34 1	38 6 ± 8 6	8 0 ± 3 0
8	Hardwood leaf mull	6 5 ± 0 1	90 5 ± 4 7	1 460 ± 0 080	29 0	24	115 ± 12 3	660 ± 17 0	66 6 ± 3 9	9 5 ± 1 9
9	Hardwood grain mull	6 4 ± 0 4	15 5 ± 2 4	0 227 ± 0 010	5 0	T	35 ± 4 2	282 ± 32 0	11 2 ± 1 0	2 8 ± 0 3
10	Hardwood crumb mull	5 3 ± 0 1	27 6 ± 2 5	0 610 ± 0 050	3 5	0	37 ± 9 2	296 ± 19.0	16 5 ± 1 9	3 3 ± 0 2

Although certain types of organic remains were shown by the analyses to be consistently high in their content of nitrates and ammonia, the figures reported in table 3 cannot be regarded as having more than a relative value, because the amounts of these compounds vary greatly with the season and with other conditions. Because of this variability, medians instead of arithmetical means are reported for these two factors.
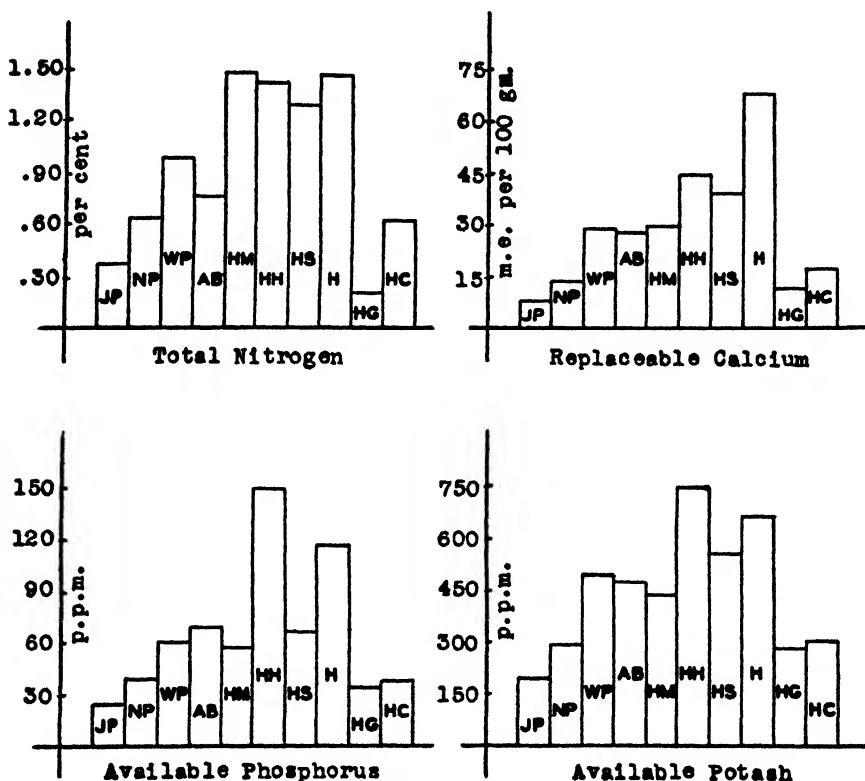
FIG. 2. CONTENTS OF NITROGEN, CALCIUM, PHOSPHORUS, AND POTASH IN DIFFERENT TYPES OF ORGANIC REMAINS FROM UPLAND FOREST

JP—jack pine duff; NP—Norway pine duff; WP—white pine duff; AB—aspen-birch duff; HM—hemlock duff; HH—hardwood-hemlock duff; HS—hardwood-spruce-fir duff; H—hardwood duff; HG—hardwood grain mull humus; HC—hardwood crumb mull humus.

Figure 2 presents graphically the contents of nutrients found in different kinds of organic matter.

With respect to the needs of nursery practice, organic remains should be classed as follows: Hardwood-hemlock duff has a fertilizing value unsurpassed by that of any other type of organic remains in the Lake States region. Hardwood-spruce-fir duff, hemlock-yellow birch duff, and hardwood leaf mull duff approach in quality the hardwood-hemlock duff. White pine duff has a decidedly lower fertilizing value than the previous type; nevertheless, it is a

very satisfactory material when applied in sufficient quantity. Aspen-white birch duff is similar to white pine duff. Norway pine duff, jack pine duff, and the earth-mulls are lowest in fertilizing value; consequently, larger quantities of these materials must be used to obtain satisfactory results.

It was thought that the best growth of any species would occur when the soil was treated with duff of the same species. This did not prove to be the case, however. For instance, both white and Norway pine grew much better on hardwood-hemlock duff than on white and Norway pine duffs applied in the same quantity. Nevertheless, it should not be overlooked that the growth response of individual species to the same type of organic remains may vary somewhat, depending on the feeding power of these species at different pH values.

Materials of an acid reaction may be used in any nursery without danger of encouraging damping-off disease. The use of organic remains having a slightly acid reaction should be confined to nurseries raising hardwoods or transplant stock.

The details of the selection of organic remains for use in forest nurseries are discussed elsewhere (8).

DISCUSSION

This study shows that the chemical composition of organic remains is an important factor in the beneficial effect which they exert on the growth of forest seedlings. Field observations, greenhouse experiments, and laboratory analyses indicate that the organic remains formed under any normally developed stand of a given composition on a certain soil type can be considered as a material of a definite fertilizing value. This information, when properly interpreted, will materially reduce the need for investigation of the chemical properties of each particular deposit of organic remains that are to be used as fertilizers in forest nurseries.

The results of this study suggest that the classification of the organic remains of upland forests can be of no practical significance unless it takes into account the characteristics of the underlying mineral soil, such as its profile, mineralogical composition, texture, reaction. and ground water level. For instance, the white pine duff formed on a well-drained, morainic sandy loam of granitic origin has a fairly high content of nutrients. On the other hand, the same duff formed on sandstone outcrops has only a negligible fertilizing value.

SUMMARY

The nutritive properties, base exchange capacity, and reaction of the important morphological varieties of humus were investigated with respect to the needs of nursery practice. The results show that the organic remains formed under normally developed stands of a given composition and on a certain soil type can be considered as a material of definite fertilizing value. Hardwood-hemlock duff formed on heavy morainic soils proved to have a

fertilizing value unsurpassed by that of any other type of organic remains in the Lake States region. Hardwood-spruce-fir duff, hemlock duff, and hardwood mull duff, formed on heavy outwash or morainic soils, approached in quality the hardwood-hemlock duff. White pine duff from glacial gravelly sandy loams showed a decidedly lower fertilizing value than the previously mentioned types. Aspen-white birch duffs from the same sites were similar to white pine duff. Norway pine duff and jack pine duff developed on outwash sandy soils, as well as true mull humus from hardwood stands, were found to be the lowest in fertilizing value. The results of this study indicate that the importance of the underlying mineral soils should be taken into consideration in a classification of organic remains for practical purposes. A close correlation between the content of essential nutrients in different types of organic remains and the growth of Norway spruce seedlings when treated with these remains was demonstrated in the greenhouse.

REFERENCES

(1) Association of Official Agricultural Chemists. 1930 Methods of Analysis, ed. 3, Washington, D. C.

(2) CHAPMAN, H. D., AND KELLEY, W. P. 1930 The determination of the replaceable bases and the base exchange capacity of soils. *Soil Sci.* 30: 391–405.

(3) HARPER, H. J. 1924 The accurate determination of nitrates in soils. *J. Indus. and Engin. Chem.* 16: 180–183.

(4) HARPER, H. J. 1924 The determination of ammonia in soils. *Soil Sci.* 18: 409–418.

(5) SNYDER, E. F. 1935 Methods for determining the hydrogen-ion concentration of soils. U. S. Dept. Agr. Circ. 56.

(6) TRUOG, E. 1930. The determination of the readily available phosphorus of soils. *Jour. Amer. Soc. Agron.* 22: 874–882.

(7) VOLK, N. J., AND TRUOG, E. 1934 A rapid chemical method for determining the readily available potash of soil. *Jour. Amer. Soc. Agron.* 26: 537–546.

(8) WILDE, S. A. 1937 Selection of forest litter, duff and humus for use in forest nurseries. Wis. Agr. Col. Tech. Notes No. 16, in coöperation with Wis. Dept. Conserv.

(9) WILDE, S. A., AND CADY, J. G. 1936 Glossary of terms related to the classification of organic layers of forest soils. Wis. Agr. Col. Tech. Notes No. 13, in Coöperation with Wis. Dept. Conserv.

PLATE 1

FIG. 1. Effect of hardwood-hemlock duff as compost ingredient upon the growth of Norway pine on wind-blown sand of granitic origin in pot cultures. 1, Complete nutrient solution including the equivalent of 1,200 pounds of 5–8–15 fertilizer per acre; 2, the same solution buffered with acid sedge-moss peat at the rate of 5 per cent on a weight basis; 3, composted fertilizer made of 9 parts of sedge-moss peat and 1 part of hardwood-hemlock duff, applied at the rate of 5 per cent and carrying an equivalent of 1,200 pounds of 5–8–15 fertilizer per acre; 4, composted fertilizer applied at the same rate and made of the same ingredients as that applied to 3, but with double the amount of hardwood-hemlock duff. Photograph taken when seedlings were 2 years old.

FIG. 2. Effects on the growth of Norway spruce seedlings of application of different types of organic remains to quartz sand cultures. 1, Check; 2, moss peat; 3, sedge peat; 4, hardwood crumb mull humus; 5, Norway pine duff; 6, hemlock duff; 7, hardwood-hemlock duff. Photograph taken when the seedlings were 2 years old.

Fig. 1
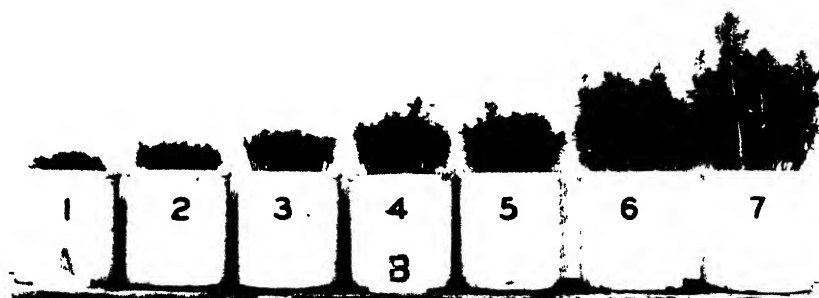
Fig. 2

A SIMPLE TYPE OF ELECTRICAL SALINOMETER FOR ESTIMATING SOLUBLE SALTS IN SOILS AND IRRIGATION WATERS

AMAR NATH PURI AND BALMOKAND ANAND

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The determination of total soluble salts in soils and irrigation waters is an important part of the routine work of a soil laboratory. The direct method consists in shaking a known amount of dry soil with a definite quantity of water, filtering, evaporating to dryness, and weighing the residue.

Complete analysis of all the salts present is sometimes required, but for most purposes merely a knowledge of the total soluble salts is sufficient for characterizing soils. In these cases methods other than the direct one result in a considerable saving of time. Of the indirect methods those based on the variations of electrical conductivity are perhaps the most popular. Their chief advantage lies in the fact that filtration of the suspension is eliminated.

Because of polarization the conductivity methods are difficult to use when applied to electrolytes. In general polarization depends on the nature of electrodes, their surface area, and the current density. If platinized electrodes of large surface area and alternating current of high frequency are used, polarization can be reduced to a minimum. The well-known method of Kohlrausch utilizing this principle involves the use of a buzzer and telephone. These methods can be made elaborate and accurate for standardizing purposes, but for routine work their use is generally restricted.

A simple, quick, and fairly accurate method for estimating total solids in soils or irrigation waters has been developed, using direct current of high voltage. In principle it is a potentiometric method, using large potential differences and a convenient type of dip electrode. By putting a high resistance in series with the electrolytic cell, the current passing through the suspension is made very small, even though the terminal potential is fairly high. The back E.M.F. developed because of polarization is small as compared to the potential difference across the electrodes of the electrolytic cell and does not produce any great error.

DESCRIPTION OF THE APPARATUS

The electrical connections of the apparatus are shown diagrammatically in figure 1. The dip electrode is made of copper and is chromium plated. The cathode and the anode are separated by a glass tube. The outer electrode is in the form of a tube of slightly greater diameter than that of the glass tube.

The distance between the outer and the inner electrode can be varied. This is useful for temperature compensation. The readings are entirely governed by the inner diameter of the glass tube, which is 1.1 cm. in this case. A temperature difference of 6°C. can be compensated by sliding the inner electrode by 1 cm. The sliding electrode is marked in degrees centigrade, the first mark being made when the distance between the tip of the electrode and the lower end of the tube is 6 cm. This corresponds to a temperature of 10°C. As the sliding electrode is taken out by 1 cm., it corresponds to 16°C., etc. It is marked up to 40°C., each centimeter being divided into six
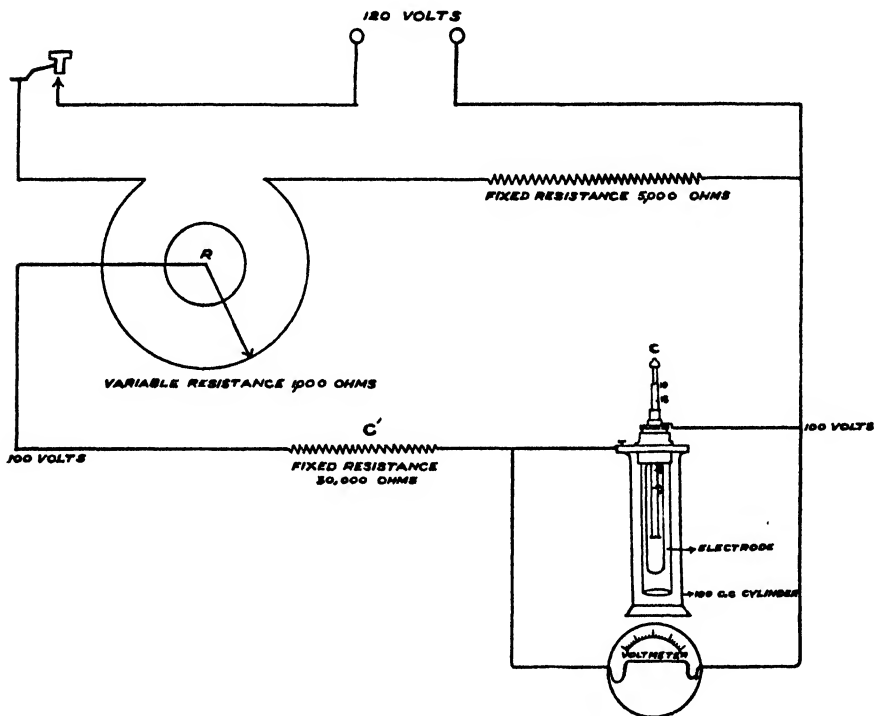
FIG. 1. ELECTRICAL CONNECTIONS IN THE SALINOMETER

parts, each representing a change of 1°. Tubes of different diameters were tried, and this size gave the most satisfactory results, though variations of 1 or 2 mm. are of no consequence.

The high tension battery is of 120 volts, and the potential is brought down to 100 volts. The sliding resistance compensates for any drop in the voltage of the high tension battery. Current from the mains can be tapped and brought down to 100 volts, but this can result in unpleasant shocks when the electrodes are touched with the hand. Shocks can be avoided by standing on a wooden platform. When a high tension battery is used, there is no danger whatsoever of shock.

Figure 1 shows that the total drop of 100 volts potential takes place along the resistance C and C', where C' is the fixed 30,000 ohm resistance and C is the resistance of the cell, which varies according to the amount of dissolved material in the water. The voltage drop across C, therefore, varies inversely as the conductivity of the electrolyte. The fixed resistance of 30,000 ohms is necessary for obtaining a fairly uniform sensitivity throughout the range of the instrument and for drawing out as little current as possible from the battery. The voltage drop across C' must always be kept constant. This is easily done by taking the dip electrodes out of the liquid and rotating the dial on the potentiometric resistance R until the voltmeter records 50 divisions.
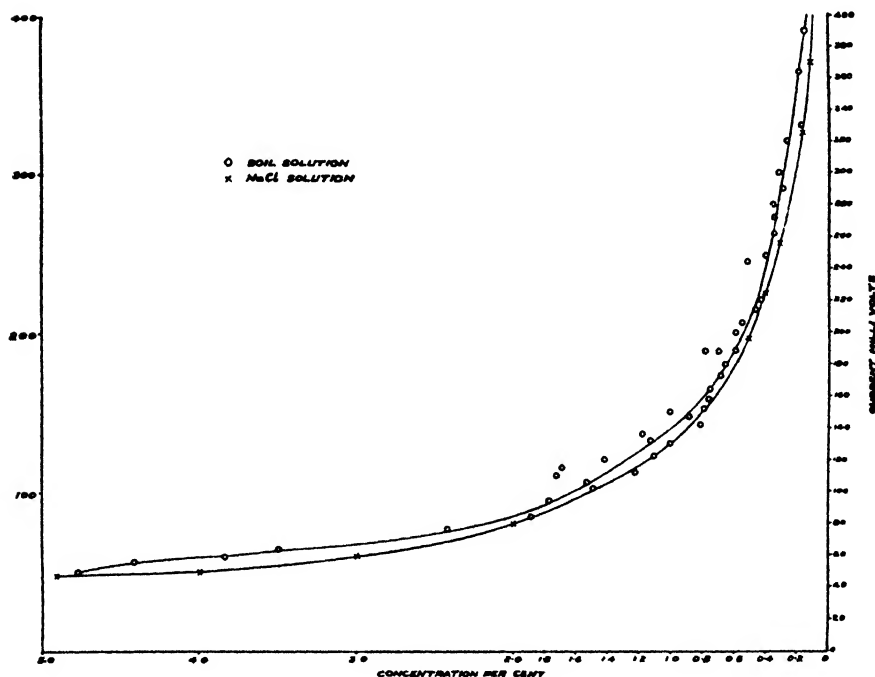
FIG. 2. RELATION BETWEEN CONCENTRATION OF SOLUTION AND CURRENT VOLTAGE

It will be seen that the resistance C' in this case is in series, and the voltmeter acts as an ammeter and actually records the current passing in terms of 50 divisions on the voltmeter. This condition is easily reproducible, and before a reading is taken with a solution, the instrument is always set at the same point by manipulating the sliding contact of the resistance R. The instrument is then ready for the examination of any unknown solution. A soil-water ratio of 1:10 is very convenient for estimating total soluble salts in soils and requires only one calibration curve for soils and irrigation waters, as shifting of the decimal point permits expressing results in parts per hundred thousand of solution or in percentages of the dry weight of the soil.

CALIBRATION OF THE INSTRUMENT

The instrument can be calibrated with a solution of sodium chloride. A number of soils with varying amounts of soluble salts were tried; the curve showing the relation between soluble salts and potential reading runs parallel to the NaCl curve. Both the curves are shown in figure 2. If the potential of the battery and the diameter of the glass tube in the electrode are kept according to the values given in this paper, no calibration is necessary, and the values given in table 1 plotted on a large scale will give a mean curve that can be used for interpolation. We have found it convenient to construct a table giving values for total solids for different values of potential for intervals

TABLE 1

Relation between conductivity and total solids in water

CONDUCTIVITY	TOTAL SDSOIL	CONDUCTIVITY	TOTAL SOLIDS
volts	*per cent*	*volts*	*per cent*
5	0.466	24	0 042
6	0 375	26	0 038
7	0.270	28	0.034
8	0.220	30	0 030
9	0.186	32	0 026
10	0.162	34	0 022
11	0.144	36	0 019
12	0 128	38	0 016
14	0 100	40	0 013
16	0 079	42	0 009
18	0.066	44	0 005
20	0 055	46	0 001
22	0 048		

of 0.2 volt. We have used such a table for the last year for thousands of samples, and frequent checkings have shown that the results compare very well with the usual method of drying and weighing the residue. It will be seen from the calibration curve that the instrument is not suitable for concentrations higher than 0.4 per cent. Such solutions must, therefore, be diluted before examination.

SUMMARY

A simple type of electrical salinometer for estimating total solids in irrigation waters and salt solutions is described. In principle it is a potentiometric method using large potential difference and a dip electrode made of copper and chromium plated. By using a high voltage the polarization E.M.F. is made small as compared to the potential difference across the electrodes.

A SENSITIVE HYDROMETER FOR DETERMINING SMALL AMOUNTS OF CLAY OR COLLOIDS IN SOILS[1]

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Michigan Agricultural Experiment Station

Received for publication April 19, 1937

The hydrometer in general use heretofore for making mechanical analysis of soils has a total range of 0 to 60 gm. a liter and is graduated in grams. Obviously a hydrometer with these characteristics does not allow a high degree of accuracy when only small amounts of clay or colloids are present. To remedy this defect, an especially sensitive soil hydrometer has been devised which has a range of 0 to 10 gm. a liter and is graduated into 0.2-gm. divisions. This special hydrometer (plate 1) is heavy, has a large, streamline bulb, comes to equilibrium quickly, and is accurate, as is shown by the data in table 1.

TABLE 1

Degree of sensitivity and accuracy of the new soil hydrometer

SOILS	AMOUNT OF CLAY FOUND IN SOIL SUSPENSIONS	
	Indicated by hydrometer	Found by evaporating to dryness and weighing residue
	per cent	*per cent*
1	3 5	3 7
2	9 8	9 8
3	1 3	1 2
4	1 9	1 7
5	7 4	7 8
6	5.2	5 0
7	9.2	9 4
8	6 5	6 3
9	2 5	2 2
10	7.0	6 8
11 (muck)	8 1	9.9
12 (ferruginous)	9 4	7.3

These data show a comparison of the amounts of soil indicated by the hydrometer in soil suspensions with the amounts that were found experimentally by evaporating the soil suspensions to dryness and weighing the residue.

Table 1 shows that the amounts of clay indicated by the hydrometer in soil suspensions are almost exactly the same as those found experimentally by

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[1] Journal article no. 295, new series, from the Michigan Agricultural Experiment Station.

evaporating the soil suspensions to dryness and weighing the residues. The only soils that show an appreciable disagreement are those that have extreme specific gravity, such as the muck and the ferruginous soil.

This hydrometer, therefore, fills a need by measuring accurately small amounts of clay or colloid in soils.

PLATE 1

A New Sensitive Hydrometer for Determining Small Amounts of Clay or Colloids in Soils
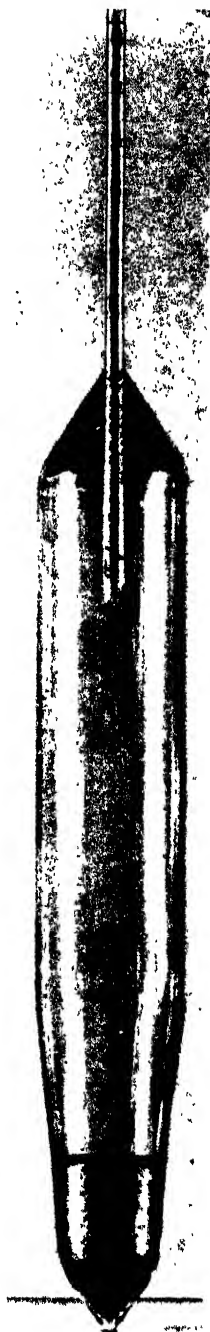
BOOK REVIEW

The Nature and Properties of Soils. A College Text of Edaphology. Third
edition. By T. Lyttleton Lyon and Harry O. Buckman. The Mac-
millan Company, New York, 1937. Pp. XIII + 392, figs. 45, tables 42.
Price $3.50

This is a thoroughly revised edition. The organization of the subject
matter is very similar to that of the preceding edition, except for a few changes
in the order of the chapters. One new chapter deals with colloids and ionic
exchange. Several other sections have been completely changed, particularly
those concerned with the transformation of organic matter and the nature of
humus, the soil profile and soil genesis, water control and soil erosion, and soil
acidity.

There are 17 chapters, bearing the following titles: A Fundamental Con-
cept of the Soil; The Supply and Availability of Plant Nutrients in Mineral
Soils; The Physical Properties of Mineral Soils; Colloidal Clay and Ionic
Exchange; The Organisms of the Soil; The Organic Matter of Mineral Soils;
Forms of Soil-Water and their Plant Relationships; Soil-Moisture Losses and
their Control; The Origin and Classification of Soil Materials; Soil Formation,
Classification, and Survey; The Nature and Utilization of Organic Soils;
The Soil Reaction—Soil Acidity and Alkalinity; Liming the Soil; The Nitrogen
Economy of Soils; Fertilizers and Fertilizer Practice; Farm-Manure and
Green-Manure; The Methods of Fertility Maintenance for Mineral Soils.

The textbook should be even more effective than the preceding volumes.
Particularly appropriate is the emphasis on the modern concepts of the forma-
tion of soils, the transformation of soil materials, and economic soil manage-
ment and soil conservation. Interest in the subject matter might be increased
if some appropriate photographs were included.

<div align="right">R. L. Starkey.</div>

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SOME RECENT OBSERVATIONS ON THE USE OF MINOR ELEMENTS IN NORTH CAROLINA AGRICULTURE[1]

L. G. WILLIS AND J. R. PILAND[2]

In the investigations of the minor element problem in North Carolina, attention has been directed largely to the study of the mechanism of the effect of these elements. Fortunately for the development of the work there are extensive areas of cultivated soils where the use of copper and manganese is beneficial and where recently a response to boron has been noted. Symptoms considered characteristic of a zinc deficiency have been observed, but as the need for this element does not appear to be acute no attempt has been made to study the zinc problem specifically.

At the outset, the effects of these elements in correcting plant abnormalities evidenced by visible symptoms were considered indicative of a deficiency in accordance with the generally accepted interpretation of prior research. As the work progressed, however, it became apparent that in many places the effect of the minor elements was dependent upon the character of the soil or other environmental factors, some of which are related to the common usage of fertilizers and to other details of soil management.

It is becoming increasingly evident that the interpretation of the results of ordinary fertilizer experiments is leading to cumulative errors in practical applications. There can be little doubt that adherence to any form of experimentation does not necessarily insure a sound basis for practical conclusions and that many of the observations relative to the effects of the minor elements support an opinion that current fertilizer practices, although derived largely from experimental data, are not only inefficient but excessive in cost. No attempt will be made to discuss this phase of the soil fertility problem in detail, but attention is directed to the possible significance of the results obtained with the minor elements relative to the functions of some of the major constituents of fertilizers.

A fundamental basis for much of the work with the minor elements is provided by the results of investigations of the oxidation-reduction equilibria of highly organic soils (8). As these results have been analyzed, a fairly satis-

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251

factory working hypothesis of general applicability has been developed.   Evidence has been accumulated leading to a conclusion that the principle applies to all soils, although serious soil defects will be found in relatively small areas.

It is not necessary to present a detailed discussion of the data on oxidation-reduction.   A conclusion has been reached that organic matter constitutes a source of potentially reductive material.   This is converted to an active form in the process of decomposition by soil microorganisms.   In extreme cases the reductive effect increases the solubility of iron to toxic concentrations.   Apparently atmospheric or molecular oxygen is not sufficiently active to prevent this excessive reductive effect, and the intervention of a catalyst is necessary to prevent injury to plants.   Tentatively it is suggested that the reaction can be expressed by the following equations:

$$O_2 + 2H^+ + \text{reductant} \rightarrow H_2O_2$$

$$H_2O_2 + \text{catalyst} \rightarrow H_2O + O$$

This implies that active oxidation in aerated soils is initiated by reduction.

MANGANESE DEFICIENCY

It has been observed in North Carolina that manganese deficiency, as evidenced by the symptoms of chlorosis, is limited almost exclusively to soils containing appreciable amounts of organic matter (7).   It has been generally assumed that managanese deficiency is caused by liming, and locally the limiting pH is approximately 6.2.   From the solubility data, however, it is evident that manganese in soils cannot be made unavailable by reducing the acidity only to this degree.   There is conclusive evidence (5) that manganese becomes unavailable only by conversion to the higher oxides, and the limiting pH value therefore represents only the point above which these oxides are insoluble.

From another angle, it is evident that manganese cannot become unavailable in strongly reductive soils, but, on the other hand, the correction of such reductive conditions by the use of a catalyst will probably promote a manganese deficiency.

COPPER AS A CATALYST OF OXIDATION

In research with a muck soil, tests of the effect of copper were made.   With corn in field plats on soil that had been cultivated for more than 20 years the response to copper sulfate was not pronounced.   When, however, it was realized that soluble iron was the probable cause of crop injury (2), attention was directed to the generally recognized principle that the major limiting factor in crop production on acid soils is the solubility of aluminum.   From the similarity of the chemical properties of iron and aluminum it was assumed that copper would be of most benefit to those crops that are intolerant of acidity on upland soils, since aluminum is not very soluble in organic soils (4).   Some tests showed that flax would not produce a crop on a muck soil except

where copper was applied. The growth of soybeans and lettuce was markedly benefited by copper on several organic soils including some that are classified as sandy loams, but in several cases it has been observed that manganese sulfate will produce an effect similar to that of copper, although with lettuce the manganese is not so efficient a corrective as is the copper (pl. 1).

The conditions under which manganese produces these results are not clearly defined. It is apparently impossible to determine the critical reductive intensity of a soil by laboratory methods, but from general principles it can be assumed that manganese toxicity may occur on reductive soils of high acidity. On the other hand, the use of copper probably will control the toxicity of manganese in soils of moderate acidity, but, according to the same principle, copper should aggravate a manganese deficiency where the pH of the soil exceeds the limiting value.

Some evidence of this associative effect is offered in the results of an experiment on Portsmouth sandy loam. The results involve other factors and will be discussed later.

In all the work with copper it has been found necessary to make the application before injury to the crop has developed, usually before planting. The proper placement of the copper application is determined by a principle that can be derived from the general hypothesis. Any material having the effect of stimulating the growth of soil microorganisms would increase the degree of reductiveness. From the evidence that phosphates on a North Carolina peat soil caused an injury to soybeans, a conclusion was drawn that this was evidence of a deficiency of phosphorus for the soil organisms in the unfertilized soil and therefore of a deficiency for crops. This deficiency could be corrected, however, only where the reductive effect was controlled, as by the use of copper. Evidence to support this assumption is available from field plats[3] although the beneficial effect of phosphate where copper is added is not demonstrated (pl. 2).

It can be concluded, however, that the locus of injury is primarily in the zone of fertilization and that the copper should ordinarily be applied with the fertilizer.

<center>BORON DEFICIENCY</center>

Attention was directed to the possibility of a widespread occurrence of boron deficiency by the observation of a leaf deformity in fields of romaine (pl. 3, fig. 1). Soil from one badly affected area was taken to the greenhouse, where it was found impossible to reproduce the characteristic symptoms in pot cultures. It was noted, however, that the plants grown without additions of borax to the soil were subject to severe wilting during hot days.

During the following season, numerous tests were made of the effect of borax at rates ranging from 1 to 20 pounds an acre on a variety of soil types.

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[3] The experimental work on the associative effect of copper and phosphate is credited to W. H. Rankin.

Three cases of a definite response were noted. One was found on heavily limed Norfolk sand with alfalfa, and two occurred with romaine on organic sandy loams that had also been limed liberally. The last two soils had previously received an application of copper sulfate; on one soil, borax applied at a rate of 2 pounds to the acre eliminated the characteristic leaf deformity except where a heavy application of calcium sulfate was made, and the results obtained on the other soil duplicated those of the pot experiments. The season was abnormally dry, and the crop wilted severely during the day on areas to which no borax was applied. No wilting occurred at any time where borax was added (pl. 3, figs. 2 and 3).

On the second field only 2 per cent of the plants with borax showed symptoms characteristic of manganese deficiency, whereas more than 60 per cent of the plants without borax showed the symptoms. This unexpected effect of borax introduced a complication of the boron problem. An assumption, based on published data (3), was made that a boron deficiency not acute enough to produce any typical symptoms might be evidenced by defective translocation of carbohydrates from the leaves to the roots of the plants. Malnutrition of the roots might be serious under these conditions, but it is also possible that in subacute cases of boron deficiency the normal excretion of organic material from plant roots does not occur and, therefore, that the roots do not exert their normal solvent effect on the soil. No attempt has been made to identify the chemical reactions of this solvent effect, but the evidence supports a conclusion that it is largely a process of reduction. It is equally possible, however, that the results obtained in the field were not caused primarily by a solvent action at the zone of contact between the roots and the soil but were due only to better translocation of manganese from the roots to the leaves.

Soil from the field in which this observation was made was taken to the greenhouse for verification of the observation. In addition to the treatments with borax and manganese that were indicated, the effect of spacing of seedlings was to be determined. It has frequently been noted that soybeans planted thickly, appear above ground more promptly and for a time are more thrifty than those that are planted at intervals. It was considered possible that this might be a result of greater solvent effects of closely associated roots. The experiments, conducted in duplicated 1-gallon pots, included the untreated soil with seeds planted closely in the middle. Under the same conditions, seeds were planted at about 2-inch intervals, and the latter spacing was repeated in the soil to which borax or manganese was added. Germination and growth were normal in all pots except those in which the seeds were spaced and no treatment was added. In the latter pots, none of the 14 seeds planted appeared above ground; they germinated but became diseased (pl. 3, fig. 4).

This susceptibility of plants to disease on manganese-deficient soils has been observed before (9), but the cause has not been determined. It is very significant that the manganese in this case produced a favorable effect before the need for this element in photosynthesis could have developed and also that

boron gave a response which was not associated with the commonly observed symptoms of a deficiency.

Further work on this detail of the minor element problem will be necessary before any definite explanation of this effect of boron can be offered.

Although these results are interesting, their significance is greatly increased when they are examined in the proper perspective. One interpretation suggests the possibility that any occurrence of a moderate degree of manganese deficiency may be indicative of a boron deficiency. Another, of greater general application, relates to the nature of the solvent effect of the roots. If this is due to a reduction process it would indicate a relation between boron deficiency and microbial activity in the rhizosphere (6). The boron problem therefore assumes the characteristics of the reductive organic soils and, depending upon the intensity of the reductive effect of the root exudates, may involve all of the features of the latter.

Copper-boron relationship

Evidence in support of such a conclusion is available in results showing the effect of some of the minor elements on the early growth of tomatoes. The soil was Portsmouth sandy loam, considered one of the most productive of the lower Coastal Plain when it is suitably fertilized. The pH was 5.4. An application approximately equivalent to 1,000 pounds of a 3-8-6 fertilizer in the plant row was mixed with the soil together with managenese, copper and zinc sulfates, and borax at rates respectively equivalent to 4, 2, 4, and 2 pounds an acre. Preliminary experiments had indicated that copper, manganese, and boron together were distinctly beneficial.

In figure 1 the heights of the plants at the time of opening of the first blooms are shown. As compared with the control, which received only the fertilizer, copper was distinctly beneficial, but manganese and boron alone were injurious. No visible symptoms of any deficiency or injury were noted. Manure applied at a rate equivalent to 5 tons an acre was very beneficial but apparently was less effective than the combination of copper and manganese. The manganese when combined with copper gave an additive effect which is in accordance with the principle discussed.

The result with copper and manganese supports a conclusion that the maximum effect of copper was not obtained with the rate of application used. If, therefore, the added burden of correcting a reductive effect developed by the use of boron were imposed, the copper could not fully correct it.

Up to this point the work has been limited to a discussion of the effect of the minor elements on the organic soils. Recently, attention has been directed (1) to the possibility of using copper profitably for cotton and tobacco on sandy soils. Although the methods used in calculating the value of the crop increases are subject to question and many of the yields reported are too low to be profitable under any conditions, there is some valid evidence of a response to copper.

Copper-potassium relationship

Experiments with cotton have been conducted during the current year with copper and other minor elements on soils where cotton rust has been severe in the past. The choice of this phase of the problem was made because of the similarity between the effects of several soil treatments on this disease and on the general productiveness of the organic soils. In both cases phosphates alone are injurious, and potash and manure are beneficial.

The results that can be reported at this time are not conclusive. On one field a distinct response to copper was evident in the appearance of the plants. Elsewhere the response to copper has been negligible. It is pertinent, however, to state that potash supplying up to 50 pounds of K_2O to the acre in addition to 12 pounds added in the fertilizer applied before seeding has not
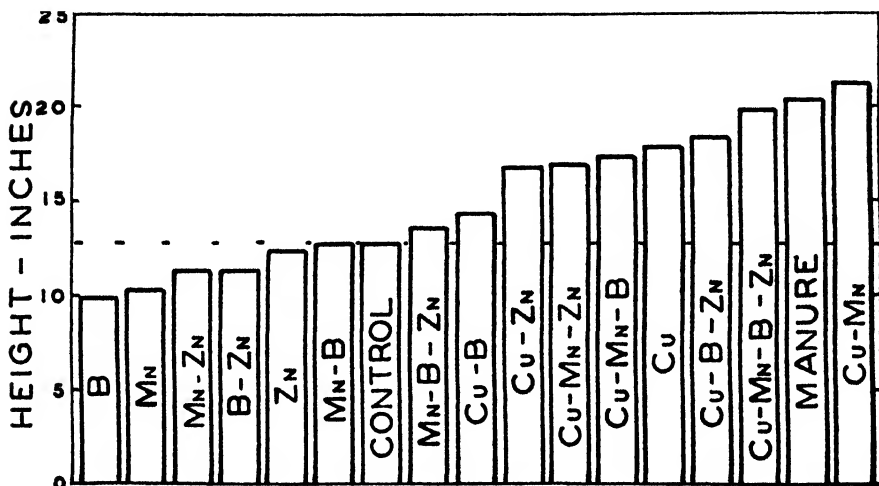
FIG. 1. HEIGHT OF TOMATO PLANTS AS AFFECTED BY COPPER, MANGANESE, ZINC AND BORON

controlled cotton rust to a satisfactory degree in all areas. Apparently the potash deficiency problem involves the correction of an unidentified soil defect which is distinct from the nutrient function of potassium. It will be necessary to determine the nature of this defect before a rational corrective treatment can be designed. It is apparent, however, that neither potassium nor copper is a specific remedy for cotton rust.

RELATION OF MINOR ELEMENT REQUIREMENTS TO EFFECT OF MAJOR NUTRIENTS

It is obvious that none of the results of field experiments from which the phosphate and potash requirements of crops have been determined are accurate for soils where the minor element problem is involved. Expediency requires, however, that they be accepted until they can be revised. Subject to the same limitation, methods of use of some of the minor elements in fer-

tilizers can be proposed, but these should be extensively tested before the formulas including them are offered for general sale.

In a summary of the fragmentary evidence of the effects of the minor elements, the following items are of practical significance. For convenience they are stated as facts, although some of them may be debatable.

An oxidation-reduction equilibrium governs the solubility of iron in the soil. With too great a reductive intensity the iron reaches toxic concentrations. Soil organic matter and organic exudates of roots systems act as sources of reductive material which is activated by microörganisms. Copper and, in some places, manganese correct the reductive effect. Copper will reduce manganese toxicity but will increase the severity of manganese deficiency. Calcium will increase boron requirements and possibily the tolerance of plants to boron. Potash has a non-nutrient effect similar to that of copper. Phosphates increase the reductiveness of soils by stimulating the growth of microörganisms.

In perfectly developed systems of fertilization the condition of the soil should be standardized especially with respect to the pH. As this is impossible, the nearest approach is through the use of non-acid-forming fertilizers. These are almost certainly essential to the successful use of the minor elements in the fertilizer formulas.

One urgent need for information cannot be ignored. This relates to the effect of some of the minor elements on the quality of crops and on alternate crops in rotation. Cigarette tobacco, in particular, is graded for sale by inspection, but the continued success of any producing area depends upon the final test of smoking quality. Cotton is very subject to rust when grown on soils that are limed to a degree suitable for peanut production, and although the minor elements may be favorable for the cotton their effects upon peanuts are not known. Without definite evidence that the influences will not be harmful it will be inadvisable to extend the use of the minor elements indiscriminately. On the other hand, it can be predicted with considerable confidence that the use of the minor elements will widen the range of adaptability of crops to soils hitherto limited in this respect and thereby permit of a greater diversification of agriculture.

SUMMARY

Evidence derived from laboratory, greenhouse, and field experiments indicates that copper, manganese, and boron may function as soil amendments or plant nutrients depending on soil and climatic conditions.

Oxidation-reduction reactions in the soil are governed by microbial reduction and oxidation by means of catalysts.

Manganese deficiency is associated with an oxidation-reduction reaction in which organic matter is involved and in which the limiting pH value represents a condition under which the higher oxides are insoluble.

Copper acts primarily as a catalyst of oxidation in organic soils, the major beneficial effect being due to a regulation of the solubility of iron within the

the limits of the tolerance of crops.   Manganese has a catalytic effect similar to that of copper but is less efficient.

Phosphates increase the injury factor of organic soils which is controlled by copper; therefore copper sulfate should ordinarily be applied with the fertilizer.

Boron deficiency is widespread in North Carolina soil.   The symptoms range from characteristic deformities of the plants to abnormal tendencies to wilt in dry seasons.   Root malnutrition and defective solvent action by roots are associated with boron deficiency.   There is evidence that the reductive intensity of the rhizosphere increases as a consequence of supplying boron to a deficient soil and that copper may be needed to correct this condition.

Under some conditions potassium will produce an effect comparable to that of copper.   This is apparently due to a non-nutrient function of potassium that is associated with an unidentified soil defect.

The indiscriminate use of the minor elements is not recommended without further experimentation in the field, although some crops in rotations may be benefited.

REFERENCES

(1) CHURCHMAN, W. L., RUSSELL, R., AND MANNS, T. F.   1936   Copper sulfate as a plant nutrient and soil amendment.   *Crop Protec. Digest* 55: 1–16.

(2) HOFFER, G. N.   1926   Testing corn stalks chemically to aid in determining their plant food needs.   Purdue Univ. Agr. Exp. Sta. Bul. 298.

(3) JOHNSTON, E. S., AND DORE, W. H.   1929   The influence of boron on the chemical composition and growth of the tomato plant.   *Plant Physiol.* 4: 31–62.

(4) PIERRE, W. H., POHLMAN, G. G., AND McILVAINE, T. C.   1932   Soluble aluminum studies: I. The concentration of aluminum in the displaced solution of naturally acid soils.   *Soil Sci.* 34: 145–160.

(5) PIPER, C. S.   1931   The availability of manganese in the soil.   *Jour. Agr. Sci.* 21: 762–779.

(6) STARKEY, R. L.   1931   Some influences of the development of higher plants upon the microörganisms of the soil: IV. Influence of proximity to roots on abundance and activity of microörganisms.   *Soil Sci.* 32: 367–393.

(7) WILLIS, L. G.   1928   Response of oats and soybeans to manganese on some coastal plain soils.   N. C. Agr. Exp. Sta. Bul. 257.

(8) WILLIS, L. G.   1934   Some potential changes induced by liming suspensions of a peat soil.   N. C. Agr. Exp. Sta. Tech. Bul. 47.

(9) WILLIS, L. G., AND MANN, H. B.   1930   Manganese as a fertilizer.   *Amer. Fert.* 72: 21–24.

(10) WILLIS, L. G., AND PILAND, J. R.   1934   The influence of copper sulfate on iron absorption by corn plants.   *Soil Sci.* 37: 79–83.

PLATE 1

INFLUENCE OF COPPER SULFATE ON ROMAINE AND BOSTON LETTUCE

FIG. 1. Romaine on soil without copper sulfate.

FIG. 2. Romaine on soil to which has been applied 50 pounds of copper sulfate an acre.

FIG. 3. Characteristic appearance of Boston lettuce on soil requiring an application of copper.

FIG. 4. Boston lettuce from a row to which copper sulfate was applied before planting. This row is adjacent to that in which was grown the plants shown in figure 3.

Fig. 2

Fig. 3   Fig. 4

PLATE 2

Fig 1 Foreground (between stakes), soybeans fertilized with phosphate; background, soybeans without fertilizer

Fig. 2 Right foreground, soybeans fertilized with 200 pounds of superphosphate an acre, background, soybeans fertilized with 200 pounds of superphosphate and 50 pounds of copper sulfate an acre.

FIG. 1

FIG. 2

EFFECT OF BORON ON ROMAINE AND SOYBEANS

FIG 1 Leaf deformity of romaine suggestive of a boron deficiency

FIG 2 Romaine plants receiving borax did not wilt during a prolonged dry period

FIG 3. Romaine plants to which no borax was applied wilted severely during the same prolonged dry period to which plants in figure 2 were subjected

FIG 4 Soybeans planted in the middle of the pot of soil 1 grew normally Those that were spaced, 2, failed to grow on the untreated soil but grew well where borax, 3, or manganese, 4, was applied.

PLATE 5

L. G. WILLIS AND J. R. PILAND

FIG. 1

FIG. 2

FIG. 3

FIG. 4

EFFECT OF LIME ON THE AVAILABILITY AND THE FIXATION OF POTASH IN SOILS[1]

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Some investigators are of the opinion that applications of lime increase the availability of the native potash of the soil to plants, and others hold the opposite view. It is known that under wetting and drying conditions some soils will change large amounts of applied potash to a relatively insoluble form (24) whereas others will change little or none. This fixation of applied potash may have an influence on its utilization by plants. The purpose of the experiments reported in this paper is to determine the effect of lime on the availability of the native potash and on the fixation of applied potash in certain soils.

MacIntire et al. (12, 13, 14), Jenny and Shade (9), Dean (3), Lamb (11), and Volk (24) have reviewed the literature dealing with the various phases of the subject.

Availability and fixation have been measured in two ways, namely, by determining the exchangeable potash[2] and by determining the amount of potash removable from the soil by the Neubauer method (15). Gedroiz (4) and Hoagland and Martin (8) have shown that non-exchangeable potash may be available to plants, and Lamb (11) has pointed out that no exact relation was evident between the amount of exchangeable potash in the soils he studied and the response to potash fertilization. What the plant can extract from the soil is the real test of availability. Despite the fact that there are objections to using the replaceable potash method as a measure of availability it seemed to be the best of the purely chemical methods for this purpose. The Neubauer method, which uses the roots of plants to extract elements from the soil, has been highly regarded especially by European workers as a method of determining available potash in soils.

As the nature and amount of colloids in the soil may influence markedly the availability and fixation of potash, it was considered necessary to make some measurements on the colloids of the soil. The amount of colloids was deter-

[2] In this paper exchangeable potash is considered as the total amount of potash removed from the soil in the leaching process and includes both the water-soluble and the exchangeable potash.

TABLE 1

Fertilizer and manure treatments, pH value, colloids, carbon, and potash of the soils from the plats of block B

PLAT NO.	TREATMENT PER ACRE*, 1908–1932	UNLIMED pH	LIMED pII	COLLOIDS Unlimed per cent	Limed per cent	CARBON Unlimed per cent	Limed per cent	K₂O PER 100 GM. SOIL† Neubauer method‡ Unlimed mgm	Limed mgm.	Replaceable Unlimed mgm.	Limed mgm.
1	No treatment	5.75	6 91	....	...	..	...	5 4	6 1	....	. .
2	1,600 lbs. nitrate soda, 75 lbs. dried blood	5.86	6 88	....	....	....	...	5.4	5 4	.....	. .
3	3,900 lbs. superphosphate	5.67	6 70		. .		.	2 4	3.6	. . .	.
4	1,335 lbs. muriate of potash	5.60	6 89	34 3	35 3	1.15	1.23	7.9	5 4	4 93	5 18
5	1,525 lbs. nitrate of soda / 300 lbs. fish scrap / 3,776 lbs. superphosphate	5.79	6 77	. .	...	.	.	3 6	3.6	.....	....
6	No treatment	5.72	6 93	31.3	33 2	1.28	1.07	3.6	3 6	3 52	3.17
7	3,900 lbs. superphosphate / 1,335 lbs. muriate of potash	5.82	6.84	36.0	34.0	1 51	1 13	2 4	6.1	4 32	3.73
8	1,675 lbs. nitrate of soda / 1,335 lbs. muriate of potash	5.96	6 75	..	..	..	.	3 0	6 2	..	. .
9	1,599 lbs. nitrate of soda / 75 lbs. dried blood / 3,900 lbs. superphosphate / 1,335 lbs. muriate of potash	5 79	6 80	. .		. .	.	5 4	3 6	..	.....
10	3,150 lbs. nitrate of soda / 100 lbs. dried blood / 7,300 lbs. superphosphate / 2,550 lbs. muriate of potash	5.74	6.65	34 0	36.0	1.49	...	5 4	6 2	4 51	5 63
11	No treatment	5 67	6 75	32 3	31 3	1 32	1 23	5 4	5 4	3 26	3.46
12	400 lbs. nitrate of soda / 100 lbs. dried blood / 50 lbs. ammonium sulfate / 1,900 lbs. superphosphate / 1,335 lbs. muriate of potash / 1,880 lbs. basic slag	5 79	6 87	30 7	29 6	1 49	1 39	5 4	3 6	3 19	3 70
13	176 lbs. calcium cyanamid / 750 lbs. superphosphate / 3,000 lbs. rock phosphate / 915 lbs. muriate of potash	5 93	6 72	..	....	....	...	6.1	6.7	.. ..	....
14	750 lbs. superphosphate / 65 tons farm manure	5.69	6 65	33.2	32.2	1.56	1.62	7.3	4 8	4.71	4 65
15	130 tons farm manure	5.71	6.77	37.3	33.2	1.62	1.61	7.3	7.3	.....	6.37
16	No treatment	5.67	6.63	39 3	35.2	1.41	1.32	3.6	4.8	5.14	4.76

* Basic slag and rock phosphate were discontinued in 1925. Dried blood, ammonium sulfate, and calcium cyanamid have been applied since 1925.

† Oven-dry basis, 105°C., 18 hours.

‡ Neubauer values obtained through the courtesy of S. D. Gray, N. V. Potash Export My., Inc., Baltimore, Md., and of S. F. Thornton, Indiana Agricultural Experiment Station.

mined by the Bouyoucas method (2), the amount of organic matter by the Allison method (1), and the pH values by means of the hydrogen electrode using a 1:2 soil-water ratio. All the pH determinations were made on air-dried soil, with the exception of the samples from block B, which were made on field-moist soil.

EXPERIMENTS WITH SOILS FROM BLOCK B

Schuster (18) obtained results from the long-time fertilizer experiments on the Delaware Experimental Farm (Sassafras silt loam), Newark, Delaware, which caused him to conclude that lime was of more value to certain crops when used with muriate of potash than when used without muriate of potash. That conclusion suggests that lime may have some effect on the availability of the potash in this soil. As the fertility plats have had regular potash and lime treatments since 1908, it was thought that tests on the soil from these plats might throw light on the question of potash availability.

Soil samples were taken from block B in the late summer of 1932 in the manner described in a previous publication (6). The block was in corn at the time of sampling. The rotation used on this block from 1908 to 1911 was corn, oats, wheat, and timothy and clover; in 1912 this was changed to corn, soybeans, wheat, and timothy and clover. The latter rotation is still in use.

The limed parts of the plats of this block received hydrated lime (analysis not known) at the rate of 2,000 pounds an acre in 1908 and 1916 and 1,500 pounds an acre in 1912, 1920, and 1924, a total of 8,500 pounds an acre. A summary of the total amount of fertilizers and manure applied is given in table 1. Detailed information about the fertilizer treatments of this block is given in other publications (18, 19).

After 100 gm. of soil was leached with 750 cc. of neutral normal ammonium acetate, the exchangeable potash in the extract was determined by the cobaltinitrite method, titrating with potassium permanganate as described in a previous publication (7).

The results for the various determinations are given in table 1.

DISCUSSION OF THE RESULTS FOR BLOCK B

The field applications of lime have changed the reaction of the soil to about the same extent over the whole block. The colloidal content is comparatively uniform, as is also the carbon content. Even plats 14 and 15, to which farm manure was applied, contain very little more carbon than the other plats, indicating that the farm manure applied has undergone rather complete biological oxidation. The difference in the amount of potash removed from the limed and the unlimed parts of the unfertilized plats by the Neubauer method is very slight. If lime affects the availability of the native potash in this soil the Neubauer results for the unfertilized plats give little indication of it. Lime has had no consistent effect on the Neubauer values of the fertilized plats. Liming likewise has had no great effect on the results obtained from the

unfertilized or fertilized plats by the replaceable potash method. Of the potash applied to these plats comparatively little appears to have accumulated in a form removable from the soil by the Neubauer or by the leaching method. This is in agreement with the results obtained by Thornton (23) on Indiana soils. In view of the fact that the total amount of potash applied was not great and that the rate of application was reduced in 1925, such results are not surprising. This soil is rather deficient in potash, and it is probable that the crops grown have removed more potash than has been applied. Snider (20) obtained lower Neubauer and lower replaceable values on heavily limed fertilized plats than on unlimed fertilized plats, and Schollenberger and Dreibelbis (16) obtained lower exchangeable values for potassium on the limed parts of fertilized plats than on the unlimed parts. These authors suggest that the lower potash values which they obtained may have been due to the larger yields and the consequent greater utilization of potash where lime was applied. Published results (18, 19) indicate that lime has had no great effect on the yields of crops grown on this soil; hence it might be expected that the plant consumption of potash would be about the same for the limed and the unlimed parts of the plats of block B. How much available potash may have been lost through drainage is, of course, not known. It seems reasonable to conclude from the experiments on block B that if lime has had very much influence on the potash in this soil, the effect has been hidden by factors not controlled under field conditions.

LABORATORY EXPERIMENTS

Laboratory experiments were conducted in order to overcome the differences which might be produced by unequal crop removals and drainage losses under field conditions.

The soils used in these laboratory experiments were Sassafras silt loam taken from the unlimed parts of the unfertilized plats of block B, Chester loam from northern Delaware, and Norfolk sand from southern Delaware. The pH values, colloidal content, and carbon content of these soils are given in table 2.

These soils were passed through a 2-mm. sieve (the material that would not go through being discarded), treated as indicated in table 4, and put in unsealed glass jars. The calcium hydroxide and potassium chloride applied were c.p. compounds. The treatments were made about August 1, 1934. Immediately after treatment, the moisture content of the Sassafras and Chester soils was brought to 40 per cent by weight and that of the Norfolk soil to 25 per cent. Thereafter the moisture was generally kept at 25 per cent for the Sassafras and Chester soils and at 20 per cent for the Norfolk soil. Once during the fall all soils were allowed to air-dry completely, and on several occasions they were allowed to dry much below the usual moisture content; thus moisture conditions that might be found under field conditions were approached. In December 1934 the soil in each jar was taken out, mixed by hand, and

replaced, and the process of maintaining the moisture of the soils was continued. A few days before February 14, 1935 the soils were allowed to dry considerably so that they could be handled more easily. The amount of moisture in the soils of the different jars on February 14, as well as the amount of moisture in samples of soil taken from the same jars and allowed to air-dry completely, is given in table 4. On February 14, the Neubauer determinations were begun, and at the same time the soils were leached for the determination of replaceable potash. The method used for determining the replaceable potash of the soils in the laboratory experiments will be discussed first.

Volk and Truog (25) indicated that in extracting soil with calcium acetate the calcium interfered in the potassium determination with sodium cobalti-

TABLE 2

The pH values, colloidal content, and carbon content of Sassafras silt loam, Chester loam, and Norfolk sand

SOIL	pH	COLLOIDS	CARBON
		per cent	*per cent*
Sassafras silt loam ..	5 28	34 5	1 31
Chester loam... ..	5 60	35 5	1 36
Norfolk sand	6 14	7.1	0.35

TABLE 3

Effect of a large quantity of calcium in the ammonium acetate leachate of Chester loam on the recovery by the cobaltinitrite method of added potassium

DETERMINATION	K ADDED	K FOUND	ADDED K RECOVERED
	mgm.	*mgm.*	*mgm.*
1	. .	3.44	.
2		3 49	. .
3	5 24	8 70	5 23
4	5 24	8 63	5 16

nitrite. In this set of experiments 16,000 pounds of calcium hydroxide an acre was applied on some of the samples (table 4), and the ammonium acetate leachates from those samples probably contain an appreciable amount of calcium acetate. To test the accuracy of the sodium cobaltinitrite method for heavily limed soil Chester loam was treated with 16,000 pounds of calcium hydroxide an acre (2,000,000 pounds considered an acre), mixed in a ball mill for 4 hours, made up to 35 per cent moisture, and air-dried; 100 gm. was leached with 750 cc. of neutral ammonium acetate. Four 75-cc. aliquots of the leachate were taken. Known amounts of potash as KCl were added to two of them, and all were analyzed by the method used in connection with the soils from block B. The results are given in table 3. The analyses were repeated, ceric sulfate being used for the oxidation of the cobaltinitrite, with

TABLE 4

*The pH values, moisture content, Neubauer values, and exchangeable potassium of Sassafras silt loam, Chester loam, and Norfolk sand treated in the laboratory in various ways**

(Moisture determined by drying 18 hours at 105°C. Exchangeable potassium and Neubauer values are for the equivalent of 100 gm. oven-dry soil.)

JAR	MOISTURE AT TIME OF DETERMINATIONS	MOISTURE WHEN AIR-DRY	pH	EXCHANGEABLE POTASSIUM	ADDED K FIXED	NEUBAUER K VALUES	ADDED K NOT REMOVED BY NEUBAUER METHOD
	per cent	per cent		mgm.	mgm.	mgm.	mgm.
				*Sassafras silt loam*			
1	6.50	1 15	5.28	5 07	....	1 59	.....
2	7 24	1 05	5.33	4 58	. ..	0.77	.... .
3	9 79	1.14	5 46	8.09	....	6 39	....
4	10 40	1 16	5 33	33 87	0 44	25.01	7.60
5	10.04	1 12	5 70	32 36	1 65	24 12	7 86
6	13.52	1.34	5 87	7 79	. .	5.76	. ..
7	9.48	1.20	6 74	31 41	2 84	22 98	8.01
8	9.53	1.15	6 91	8 03	..	4.77	....
9	10 13	1 22	8 29	28.57	4 95	18 96	12.37
10	8 97	1 13	8 29	7.30	. .	5 11	.. ..
				*Chester loam*			
1	8 65	1.63	5.60	21.06	. ..	14 28	....
2	7 60	1.49	5.77	20 45		17 31	. .
3	9.69	1.54	5 85	23 48	.	27 06	. ..
4	10.24	1.68	5 77	45.16	4 54	39 64	13.64
5	9 99	1 60	6 15	44 41	4 19	39 03	12 66
6	10 63	1.95	6 02	22 38		25 47	. .
7	13.91	1.70	7 02	42 36	5 97	42 43	14.95
8	16.45	1 85	7 02	22 11	....	31 16	.....
9	16 91	1 78	8 10	44 60	4 14	44 41	20 20
10	10 82	1 47	8 10	22 52	.	38 39	... .
				*Norfolk sand*			
1	1.28	0 24	6 14	5.68	....	0 40	.....
2	1.51	0 21	6 12	3.44	....	0 32	.....
3	0.99	0.23	6 19	7.73	. ..	4 90	.....
4	2.10	0.24	6.04	31 22	2.73	20 88	10.25
5	2.12	0.22	7.24	29.38	3 71	21 27	10 24
6	1.46	0.28	7.36	6.87	....	5 29	.....
7	3.23	0.25	8.30	27.53	5.72	21.08	11.95
8	4.92	0.26	8.40	7.03	....	6 81	.....
9	3.44	0.24	8.46	26 08	8.34	21 44	10.75
10	2.61	0.26	8 48	8 20	....	5.97	.....

* Treatment:

All jars sieved.

Jar 1 retained in field-moist condition until experiments began; all other jars air-dried.

Jars 1 and 2 no mixing in ball mill; all other jars mixed 4 hours in ball mill.

Jars 4, 5, 7, and 9, 1,000 pounds KCl per acre (2,000,000 pounds soil), equivalent: o 26.22 mgm. K per 100 gm. soil.

Jars 5 and 6, 1,000 pounds Ca(OH)₂ per acre; jars 7 and 8, 4,000 pounds Ca(OH)₂ per acre; jars 9 and 10, 16,000 pounds Ca(OH)₂ per acre.

practically identical results. In view of these results the method used in connection with the soils from block B was adopted, the only modification being that all leachings were made on the equivalent of 50 gm. of oven-dry soil with 1,200 cc. of the acetate solution for Sassafras silt loam and Chester loam and with 1,150 cc. for Norfolk sand.

The results of the analyses are presented in table 4. They will be discussed later in conjunction with those for the Neubauer determinations.

Neubauer determinations

The procedure for Neubauer determinations has been explained by the author (5), by Thornton (22, 23), and by others. In these experiments the Neubauer procedure was very closely followed. The plants were grown in a Minnesota seed germinator with glass door as described by Thornton (22, 23), and the temperature was controlled at 20°C. Semesan Jr. was used for disinfecting the rye seed. The rye seed used in these determinations weighed about 3.2 gm. per hundred seed. This is smaller than Neubauer prescribes, but the seed were the largest available. The Neubauer experiments were conducted in duplicate.

Analysis of seedling material

Preliminary experiments indicated that the sodium cobaltinitrite method was satisfactory for determining the potash in the seedling material, hence this method was adopted. The procedure used was as follows: The plant tissue was ashed at 550°C. in a furnace, and the ash was treated with 3 cc. of 25 per cent HCl and, after the addition of a little water, evaporated to dryness on a water bath. The residue was taken up with 1 cc. 25 per cent HCl and hot water, washed into a 100-cc. graduated flask, and made up to volume. The analyses were made by the Schueler and Thomas method (17) with the filtration modification used by the author (7).

All analyses were repeated, ceric sulfate (7) being used instead of potassium permanganate. The two sets of analyses were almost identical; therefore only the results obtained with the potassium permanganate are reported. The difference in potassium content of the plant material from duplicate Neubauer dishes ranged from 0 to 1.5 mgm. The values reported (table 4) are the averages of the duplicates.

DISCUSSION OF THE RESULTS OF THE LABORATORY EXPERIMENTS

In these laboratory experiments it was considered necessary to mix thoroughly the $Ca(OH)_2$ and KCl with the soil, and a ball mill seemed the best means for doing it. Moist soil will cake in the ball mill, hence it is necessary to work with air-dry soil. Volk (24) found that drying the soil caused it to fix potash. Alternate wetting and drying increased the fixation, but one drying produced about 75 per cent of the total fixation. As a check on the effect of drying the soil before treatment, the soils in jar 1 were sieved and

retained in their field-moist condition until the experiment was begun. The replaceable potash of the soils retained in their moist condition up to the beginning of the experiments is higher in each soil. The difference is not very great except in Norfolk sand, where it amounts to about 2 mgm. The difference in Neubauer values between the air-dried and moist Sassafras silt loam and Norfolk sand is not very great, but about 3 mgm. more potassium was removed by the Neubauer method from air-dried Chester loam than from the same soil kept in a moist condition up to the beginning of the experiments.

Kelley, Dore, and Brown (10) found that grinding soil colloids increased the amount of exchangeable potash. The results for jars 2 and 3 show that for all soils mixing in the ball mill increased the amount of exchangeable potassium and the amount of potassium removed by the Neubauer method. The results are, therefore, in agreement with those obtained by the authors mentioned. It should be noted that grinding has had relatively a much greater effect on the potash of Sassafras silt loam and Norfolk sand than on that of Chester loam. Evidently there is something about Chester loam that made the grinding less effective than that of the other two soils.

The results for jars 3, 6, 8, and 10 should show the effect of the $Ca(OH)_2$ on the native potash of the soils. Lime on those samples apparently has not affected very markedly either the replaceable values or the Neubauer values of any of the soils, except that the heavier applications have considerably increased the Neubauer values of Chester loam.

Jars 4, 5, 7, and 9 were treated with KCl at the rate of 1,000 pounds per acre, which is equivalent to 26.22 mgm. of potassium per 100 gm. of soil. The amount of fixation of this applied potassium was calculated by adding the proper blank for soil to the 26.22 mgm. of potassium and from this subtracting the total amount of potassium obtained from the soil on which it was applied. Fixation values were calculated for both the leaching and Neubauer results.

The unlimed Sassafras silt loam fixed little of the applied potash as measured by the leaching method but fixed 7.6 mgm., or about 29 per cent, as measured by the Neubauer method. Increasing amounts of lime increased the fixation as measured by both methods. The maximum amount of fixation as measured by the leaching method was 4.95 mgm., or about 19 per cent, and the maximum as measured by the Neubauer method was 12.37 mgm., or nearly 50 per cent.

The unlimed Chester loam fixed potash as measured by both methods, the fixation being 4.54 mgm., or about 17 per cent by the leaching method, and 13.64 mgm. or about 50 per cent by the Neubauer method. Increasing amounts of lime had little effect on the fixation as measured by the leaching method, but the heaviest application of lime increased the fixation as measured by the Neubauer method to 20.2 mgm., or about 77 per cent. It should be noted that the heavier applications of lime increased the amount of native potash removable from the soil by the Neubauer method and at the same

time increased the fixation of the applied potash. That does not seem to be consistent.

Both methods indicated a fixation of potassium by the unlimed Norfolk sand. The leaching process gave a fixation of 2.73 mgm., or about 10 per cent, and the Neubauer method gave 10.25 mgm., or about 39 per cent. Lime increased the fixation as measured by the leaching method up to 8.34 mgm., or about 32 per cent, but it had little effect on the fixation as measured by the Neubauer method.

There seems to be no relation between the fixation and either the colloidal content or the amount of organic matter in the soil. Norfolk sand (table 2) has about one fifth the colloidal content of Sassafras silt loam and of Chester loam and about one fourth the carbon content of those soils, yet the unlimed Norfolk sand fixed more potash than the unlimed Sassafras silt loam and almost as much as the unlimed Chester loam.

The $Ca(OH)_2$ has changed the pH values of the soils, as would be expected. It may be said in general that the greatest fixation has occurred at the highest pH values. There appears to be no pH value above which fixation occurs and below which none occurs. Stewart (20) points out that some European workers have obtained results indicating that varying the pH values within the ordinary range of soils has little effect on the absorption of potash by the Neubauer method, but that above pH 8.0 or 8.3 absorption decreases. Norfolk sand is the only one of these treated soils that has a pH value as high as 8.3. In this soil no such relation held. The absorption by the Neubauer method was practically the same from pH 6.04 to pH 8.46.

SUMMARY AND CONCLUSIONS

The exchangeable potash and the Neubauer values for potash, as well as the pH, carbon content, and colloids were determined for soils from the plats of block B on the Delaware Experimental Farm. Sassafras silt loam taken from the unlimed parts of the unfertilized plats of block B, Chester loam from northern Delaware, and Norfolk sand from southern Delaware were treated in various ways in the laboratory, and after treatment the potash removable by the leaching process and by the Neubauer method was determined. From the results obtained the following conclusions may be drawn:

1. The lime applied to the plats of block B seems to have had no great effect on potash availability as measured by replaceable potash and Neubauer values. Losses through drainage and unequal removal by the crops could have concealed the effect.

2. Little of the potash applied to block B appears to have accumulated in the soil in a form extractable by the leaching and the Neubauer methods.

3. Grinding the soils in a ball mill increased the exchangeable potash and the Neubauer values.

4. $Ca(OH)_2$ applied to the soils under laboratory conditions had comparatively little effect on the native potash of the soil as measured by the leaching and the Neubauer methods, except that the heavier applications of $Ca(OH)_2$ to Chester loam increased considerably the Neubauer values.

5. The unlimed Sassafras silt loam, Chester loam, and Norfolk sand each fixed applied potash. The highest fixation of any of the unlimed soil was about 50 per cent on Chester loam as determined by the Neubauer method.

6. In general, increasing the amount of lime increased the fixation, but on this point the leaching method and the Neubauer method are not in agreement either on Chester loam or on Norfolk sand.

7. The Neubauer values for fixation are higher in all cases than those obtained by leaching. The highest fixation of the applied potash as measured by the Neubauer method was about 77 per cent with the heaviest application of Ca(OH)$_2$ on Chester loam.

8. Fixation occurred at all pH values (5.33 to 8.46) of the soils studied.

9. Fixation seems to have had no relation to the amount of colloids and organic matter in the soils.

REFERENCES

(1) ALLISON, L. E. 1935 Organic soil carbon by reduction of chromic acid. *Soil Sci.* 40: 311–320.

(2) BOUYOUCOS, G. J. 1936 Directions for making mechanical analysis of soils by the hydrometer method. *Soil Sci.* 42: 225–229.

(3) DEAN, H. C. 1936 The effects of liming on the liberation of potassium in some Iowa soils. Iowa Agr. Exp. Sta. Res. Bul. 197.

(4) GEDROIZ, K. K. 1931 Exchangeable cations of the soil and the plant: I. Relation of plant to certain cations fully saturating the soil exchange capacity. *Soil Sci.* 32: 51–63.

(5) HARRIS, HENRY C. 1928 Effect of lime on the availability of phosphorus in super-phosphate (acid phosphate). *Jour. Amer. Soc. Agron.* 20: 381–393.

(6) HARRIS, HENRY C. 1934 The influence of cropping system and fertilization on the reaction of Sassafras silt loam soil. *Jour. Amer. Soc. Agron.* 26: 29–34.

(7) HARRIS, HENRY C. 1935 A comparison of potassium permanganate and ceric sulfate for the oxidation of cobaltinitrite in the estimation of potassium in KCl solution and in ammonium acetate soil-extracts. *Soil Sci.* 40: 301–309.

(8) HOAGLAND, D. R., AND MARTIN, J. C. 1933 Absorption of potassium by plants in relation to replaceable, non-replaceable and soil solution potassium. *Soil Sci.* 36: 1–35.

(9) JENNY, H., AND SHADE, E. R. 1934 The potassium-lime problem in soils. *Jour. Amer. Soc. Agron.* 26: 162–170.

(10) KELLEY, W. P., DORE, W. H., AND BROWN, S. M. 1931 The nature of the base-exchange material of bentonite, soils, and zeolites, as revealed by chemical investigations and X-ray analysis. *Soil Sci.* 31: 25–55.

(11) LAMB, JOHN, JR. 1935 The availability of soil potassium. *Soil Sci.* 40: 365–381.

(12) MACINTIRE, W. H., SHAW, W. M., AND SANDERS, K. B. 1927 The influence of liming on the availability of soil potash. *Jour. Amer. Soc. Agron.* 19: 483–505.

(13) MACINTIRE, W. H., SHAW, W. M., AND YOUNG, J. B. 1930 The repressive effect of lime and magnesia upon soil and subsoil potash. *Jour. Agr. Sci.* 20: 499–510.

(14) MACINTIRE, W. H., SHAW, W. M., YOUNG, J. B., AND ROBINSON, B. 1936 The effects of 12-year residues of lime and magnesia upon the outgo of subsequent additions of potash. *Jour. Amer. Soc. Agron.* 28: 202–215.

(15) NEUBAUER, H., AND SCHNEIDER, W. 1923 Die Nährstoffaufnahme der Keimpflanzen und ihre Anwendung auf die Bestimmung des Nährstoffgehalts der Boden. *Ztschr. Pflanzenernähr. u. Düngung.* 2A: 329–362.

(16) SCHOLLENBERGER, C. J., AND DREIBELBIS, F. R. 1930 Effect of cropping with various fertilizer, manure, and lime treatments upon the exchangeable bases of plat soils. *Soil Sci.* 29: 371–394.

(17) Schueler, J. E., and Thomas, R. P. 1933 Determination of potassium by sodium cobaltinitrite. *Indus. and Engin. Chem. Anal. Ed.* 5: 163–165.

(18) Schuster, George L. 1924 Fifteen years of field experiments with manure, fertilizers and lime on Sassafras silt loam soil. Del. Agr. Exp. Sta. Bul. 137 (Tech. Bul. 4).

(19) Schuster, Geo. L., and Phillips, C. E. 1929 Fertility rotation experiments. *Del. Agr. Exp. Sta. Bul.* 162: 14.

(20) Snider, H. J. 1934 A chemical study of a soil under long-continued field experiments. *Jour. Amer. Soc. Agron.* 26: 946–953.

(21) Stewart, R. 1932 The Mitscherlich, Wiessmann, and Neubauer methods of determining the nutrient content of soils. Imp. Bur. Soil Sci. Tech. Commun 25.

(22) Thornton, S. F. 1931 Experiences with the Neubauer method for determining mineral nutrient deficiencies in soils. *Jour. Amer. Soc. Agron.* 23: 195–208.

(23) Thornton, S. F. 1935 Soil and fertilizer studies by means of the Neubauer method. Purdue Univ. Agr. Exp. Sta Bul. 399.

(24) Volk, N. J. 1934 The fixation of potash in difficultly available form in soils. *Soil Sci.* 37: 267–287.

(25) Volk, N. J., and Truog, E. 1934 A rapid method for determining the readily available potash of soils. *Jour. Amer. Soc. Agron.* 26: 537–546.

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THE MEASUREMENT OF PHOSPHATE FIXATION IN SOILS

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The power of soils to fix phosphates has long been recognized, but it is only in recent years, and principally through the efforts of Hawaiian workers, that the study has received much close attention in soil research. The literature is fairly extensive, but the methods of determining the fixing power are still at variance; this points to the necessity of some rapid and effective method by which phosphate fixation can be estimated and be at the same time allied to agricultural practice.

MEANING OF PHOSPHATE FIXATION

The theory of phosphate fixation is very intricate. It was formerly believed that on the addition of a soluble phosphatic fertilizer to the soil a simple chemical reaction would occur in which phosphate ions would combine with the bases in the soil and form insoluble compounds. This is the chemical theory, which is, perhaps, essentially true. There is evidence, however, that the reaction that takes place cannot be so simply explained. The quantity of soil phosphates fixed is roughly proportional to the amount of clay present. Consequently it was inferred that the colloidal complex plays the major rôle in fixation. That fixation may not be due entirely to these reactive colloids appears in the fact that the smaller the particles of soil are ground, the higher is the fixing power. Hibbard (23) and others have pointed out that the fixing power of the soil colloids varies inversely as the ratio SiO_2/R_2O_3 of the colloid. Scarseth and Tidmore (34) state that the reaction between soil phosphates and colloidal clay is virtually instantaneous, but the fact that it is not completed militates against the strictly chemical theory.

Before the physicochemical theory is considered it may be well to review the various factors influencing the rate and amount of phosphate fixation. Let us assume for the present that the reaction is chemical, that it is, in fact, due to double decomposition, despite the objections raised by Davis (9).

In order that chemical reaction can take place the soil must be in an active condition. With calcium, iron, aluminum, and other bases the reaction takes place very readily under certain conditions. Dehydrated iron oxide will not react with a soluble phosphate. Ford (14) showed that fixation in difficultly soluble form is due to the hydrated iron oxides, such as goethite, which form

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very difficultly soluble basic iron phosphates. The fixation of phosphates by iron and aluminum depends, naturally, on the degree of hydration of the oxides of these elements. It is unlikely that ferric hydroxide, $Fe(OH)_3$, exists in nature, as it is believed (2) to be an unstable compound. Of the monohydrate and the dihydrate, the former is by far the more reactive. As the monohydrate, iron exists as the compound goethite. Heck (22) considers it very unlikely that the dihydrate exists in nature. The rôle of active hydrated iron can then be limited virtually to the monohydrate. It is not stated in what form the hydrated oxides of aluminum are most active. The chemical reaction with both iron and aluminum takes place to form very insoluble phosphates. Active calcium exists usually in the form of the carbonate or of a soluble calcium salt. McGeorge and co-workers (25, 28, 30) have evidence to show that in addition to the formation of $Ca_3(PO_4)_2$, a compound not so permanently fixed as the phosphates of iron and aluminum, a very insoluble basic carbonate-apatite may be formed in calcareous soils. It has also been suggested by other investigators, and the general experience is, that the availability of phosphate is low in many soils which contain an excess of calcium carbonate. Heck (19) points out that when active calcium and aluminum are low, phosphates are fixed largely by iron, and the resulting compound is generally less soluble than the corresponding aluminum salt. Cook (6) showed that increasing additions of lime to fine acid soils consistently lowered the power of these soils to fix added soluble phosphate in a difficultly soluble form.

Effect of hydrogen-ion concentration. The condition of acidity or alkalinity of the soil is determined by the nature of the reaction equilibrium that takes place between the PO_4 ions and the soil bases. The effect of pH, however, may not be looked upon as a dominant factor, as large excesses of active iron and aluminum are believed to overshadow its influence on the products of chemical action. Soils of high alkalinity increase the calcium in the mass action equilibrium with soluble phosphates, whereas in acid soils calcium is thrown out in preference for iron and aluminum. In general we may say then that fixation in acid soils is of a far more permanent nature than that in alkaline soils. The order of stability of the three compounds varies directly, then, with the pH value. In very acid soils, iron phosphate is more stable than aluminum phosphate. Pugh (31) states that calcium phosphate is insoluble within a pH range from neutral to strongly alkaline. Brown (4) found that he could obtain a far greater amount of available phosphorus at pH 7.9 than at any other reaction and explains this by the low solubility of iron and aluminum and the little free calcium at this reaction. Heck (22) quotes Gaarder's finding that calcium phosphate is least soluble at pH 6.5. It was found further that at pH 6.0 the solubility of calcium, iron, and aluminum phosphates was the same, and that in consequence each of these bases has an equal chance in competition for the applied phosphate. In such instances the quality of the reaction would be determined simply by the relative amounts of these bases present in an active form. At its point of lowest solubility, pH 6.5, calcium

phosphate is only one third as soluble as either iron phosphate or aluminum phosphate.   From pH 6.5 upward, calcium predominates in combining with soluble phosphates.   Below this figure it is largely eliminated from the fixation equilibrium.

Hibbard (23) states that soil colloids of iron and aluminum may fix PO_4 at pH values ranging from 3 to 8.   Above pH 8 they may hydrolyze to form the soluble phosphate salt.   There appears to be no lower limit of acidity in which the fixation takes place with iron and aluminum.   Doughty (12) states that although the formation of iron, aluminum, and calcium phosphates will account for the fixation above pH 2, there is considerable fixation below this point. Davis (9) found extensive fixation occurring at pH values as low as 0.25.

Effect of exchangeable bases.   Heck (21) found that soils from which exchangeable bases had been removed fixed more phosphates in the difficultly available form than did soils containing exchangeable bases and that the increase was somewhat proportional to the exchangeable bases removed. Conversely, increased exchange capacity tends to decrease phosphate fixation, provided that the proper degree of base saturation is maintained.

Davis (9) in discussing sorption of phosphate ions states that absorption of the phosphate by the soil minerals and the formation of equilibrium complexes take place in soils artificially depleted of exchangeable bases, thus eliminating the possibility of cations of soluble salts present in the soil, or cations replaced from the soil by those present in the solution, forming precipitates with the phosphate ions.

Effect of organic matter.   Doughty (13) concludes that soil organic matter as such has only a minor rôle, if any, in the fixation of phosphorus in difficultly available form when soluble phosphatic fertilizers are applied to the soil. Natural and synthetic humus did not fix phosphorus.

Effect of time.   Many investigators have shown that phosphate fixation is a time reaction and may continue for months.   Scarseth and Tidmore (35) state that when very soluble phosphates, such as mono-ammonium or monocalcium phosphates, are added to an acid soil the fixation of PO_4 ions takes place as rapidly as the phosphate is diffused throughout the soil and comes in contact with the colloids.   Conditions in the soil, however, may be far from ideal.   The rate of fixation is, as one would expect, considerably influenced by the soil:solution ratio.   Brown and Munsell (3) showed that, after 20 months, fertilization with both rock phosphate and superphosphate had no appreciable influence on the accumulation of phosphates at a depth below 1 inch.   Hance (15) defines a high-fixing soil as one that allows the soluble phosphate to penetrate 2 or 3 inches, and a mild-fixing soil as one that allows the phosphate to travel 2 or 3 feet.   Hibbard (23) states that clay soils fix within a year all PO_4 added, whereas in a 1:1 soil-water mixture most of the fixation takes place in an hour.   Brown (4) showed that biennial surface applications of superphosphate penetrate not more than 2 or 3 inches, and perhaps much less, in 16 years.   Rock phosphate applied in the same manner was able to penetrate

more than 7 inches.   The consensus is that phosphate fixation under natural conditions is a time reaction and may proceed rapidly in some soils and slowly in others.   Under normal field conditions equilibrium is established in 1–12 months, according to Heck (20).

Effect of soil composition.   High fixation may be expected in clay soils, whereas in sandy soils the fixation is somewhat limited.   In each case soil reaction and soil composition decide the type of fixation.   It was observed earlier that fixation in alkaline soils is not so permanent as fixation in acid soils.   In acid clay soils the abundance of colloids rich in reactive iron and aluminum would appear to raise a serious problem in connection with the application of soluble phosphatic fertilizers.   Too little, however, is known of the subject to warrant emphasis on this point, and, as will be seen later, the reaction of the soil cannot be taken as a final indication of the type of fixation, although it can be safely assumed for example, that in alkaline calcareous soils, abundant in reactive calcium, the phosphates in all probability will combine with the alkaline earth carbonates.   In alkaline solutions iron hydrolyzes, liberating PO_4 ions which combine with calcium, the hydrolyzed iron remaining insoluble.   Hibbard (23) suggests that this occurs above pH 8.   Phosphorus itself fortunately does not hydrolyze to form an insoluble acid.

The rôle of soil composition, particularly the composition of the clay fraction is a very decisive factor.   Heck (22) found that most low-fixing soils are dark and that many contain lime and plentiful organic matter.   Reddish yellow or yellow soils have the highest fixing power.   This color arises from the presence of monohydrate (and perhaps dihydrate) ferric oxide, which, as remarked earlier, is highly reactive.   Hance (15) states that subsoils show higher fixing power than surface soils.   Laterites with a high content of hydrated oxides of iron and aluminum have a high fixing power.   Thus Craig (7) found that although the highly laterized soils of certain regions of Mauritius were rich in HCl-soluble phosphorus, very little of this was of immediate value to plants.

Heck (19) states that the part played by magnesium, manganese, and titanium in phosphate fixation is uncertain, except in very special cases.   Titanium phosphate is stable in acid solutions but is dispersed at pH 6.0.   Pugh (31) found that with increasing acidity of the system $TiCl_4 + Na_2HPO_4$ more P_2O_5 was retained by the precipitate.   Hydrolysis of the magnesium salt takes place in both acid and alkaline media, and hence the salt may be considered less stable than the corresponding phosphate of calcium.   Magnesium therefore offers even less of a problem in phosphate fixation than does calcium.   At any rate, the accumulation of magnesium in the soil is never very great.

The only elements in the soil that play a rôle of any importance in phosphate fixation are, therefore, iron, aluminum, and calcium.   Phosphate fixation can be easily understood on these purely chemical grounds and takes place logically in accordance with our knowledge of factors governing these simple equilibrium reactions.

The physicochemical theory. It was pointed out earlier that more recent investigators had come to the conclusion that phosphate fixation could not be explained along purely chemical lines and that there was sufficient evidence to show that a greater rôle was played by sorption. Davis (9), who has made an extensive examination of this theory, inclines toward the belief that phosphate fixation can be largely accounted for by absorption. That the process of fixation can be reversed very slowly points rather to absorption than to adsorption. Mattson (30) showed the interrelationship of adsorption of phosphate with the electrokinetic behavior of soil colloids. Ravikovitch (32) mentions four different forms of adsorption by H-soils and five forms by Ca-soils, these different forms being connected with the phenomenon of adsorption of phosphoric acid ions of different values. Adsorption of PO_4 ions, in general, proceeds on the principle of an equivalent anion exchange; in H-soils the exchange is between the PO_4 ions and the exchangeable hydroxyl ions, and in the Ca-soils the exchangeable calcium is replaced by the cations of the phosphate solution used. The first form of adsorption[2] by Ca-soils may be represented as follows:

$$Ca \diagup^{OH}_{\diagdown H_2PO_4}$$
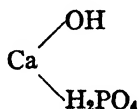
Ravikovitch thus shows that the amount of adsorption depends on the anion exchange capacity of the soil complex (which equals the base exchange capacity) and on the valence of the phosphoric acid ions which take part in the adsorption. Scarseth (33) observed that the attractive force of the phosphate anions was inversely proportional to the concentration of the hydroxyl anions, thus the increase of phosphate ions in solution with the increase of pH values may be looked upon as an anion exchange phenomenon. Davis (9), by the not unreasonable supposition that phosphate ions are strongly held by non-polar adsorption, dismisses the objection that as soil colloids are negatively charged they will not adsorb anions. He (10) inclines to the belief that the new compounds formed are complexes containing the phosphate radical HPO_4 or H_2PO_4 and the radicals K, Na, etc., in addition to the substances in the soil. The whole compound is fixed; that is to say, not only the PO_4 but the basic portion as well, combines with the soil colloids to form addition compounds or complexes, resulting in a change in the composition and structure of the colloidal alumino-silicate. According to Davis (9) the work of Ford (14) and others presents evidence in support of this physicochemical reaction.

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[2] According to McGeorge (25, 26), H_2PO_4 ions are largely absorbed by plants but this absorption will not take place above approximately pH 7.6.

VARIOUS METHODS OF INVESTIGATION

The procedure for estimating phosphate fixation generally consists in shaking the soil with a phosphate solution and determining the amount of phosphate that remains in solution after this treatment. Though under favorable conditions most of the fixation takes place within a brief period, it is not to be expected that even under laboratory conditions a final equilibrium can be established. Furthermore, various factors would tend to limit the value of this study were they not taken into consideration. Such factors as the soluble salt used, the soil:solution ratio, the pH value of the solution, the time, and the temperature must be reckoned with. It follows, then, that a uniform procedure must be ultimately sought.

Among the soluble phosphate solutions used by various workers are mono- and di-ammonium phosphate, either singly or combined; mono-calcium phosphate; mono-potassium phosphate; and phosphoric acid. The rates at which different soluble phosphatic salts are fixed varies. Thus it has been found that di-sodium phosphate is much less readily fixed than is di-potassium phosphate. The hypothetical sodium phosphate complex is assumed to be more highly dissociated than the corresponding potassium complex. According to other workers, mono-sodium phosphate is also less readily fixed than the corresponding potassium salt. Tri-sodium phosphate is less strongly fixed than mono-ammonium phosphate. It seems, then, that in the more alkaline phosphate solutions, soils have less ability to fix phosphate ions than they have in the less alkaline solutions. Workers in Hawaii recommend the use of di-ammonium phosphate adjusted with hydrochloric acid to pH 6.8 together with the addition of an electrolyte, such as ammonium chloride, to aid in subsequent filtration. It is pointed out by Hibbard that the relative fixing power of different soils is different for different phosphatic salts, thus indicating that the most effective salt for one soil may not be the best for a different soil. Such facts cannot be brought into consideration. Any laboratory method is necessarily approximate, and the ideal to be aimed at is a standardized procedure which will at the same time throw light on the practical problems of agriculture.

The ratio soil:solution plays a decisive part in the rate of fixation. Hibbard (23) states that the greater the volume of water mixed with a soil the greater will be the amount of PO_4 remaining in solution. In making a test of fixing power, the ratio of soil to water must be held constant in order to obtain comparable results with different soils.

Heck (20) prefers the use of mono-calcium phosphate to that of other phosphates, as this is the main ingredient of superphosphate. The value of this salt, however, is questionable. Heck brought the soil and solution to dryness and extracted subsequently in the cold with 0.002 N H_2SO_4. Mono-calcium phosphate is readily decomposed by a small quantity of water, forming insoluble dicalcium phosphate and free phosphoric acid. With heat the same salt is precipitated free from water, and it is difficult to know whether water

is entirely removed by the treatment with weak acid. In the usual run of experiments the unfixed PO_4 is determined in the filtrate, and the amount fixed can be determined directly by subtraction. Hibbard (23), however, remarks that if the amount necessary to give the desired concentration in the extract, instead of the amount actually fixed, is taken as a measure of fixing power, a more reliable estimate of the character of the soil is obtained.

Davis (9) used a ratio of 1 part soil to 10 parts solution and agitated the mixture for 24 hours. This ratio was used also by Ayres (1). Heck (20) preferred a ratio one tenth of this. Hance and Yuen (18) used a 1:5 ratio.

PROPOSED METHOD OF INVESTIGATION

Before the proposed method of investigating PO_4 fixation is outlined it may be well to obtain a clear conception of the term "fixed." When we say that fixation takes place do we mean that the applied phosphate has been locked up permanently by the soil or has it merely been temporarily withheld from the solution? Heck (19) makes the following divisions:

Readily available fixed phosphorus . . . . $Ca_3(PO_4)_2$
Moderately available fixed phosphorus. . . . . $AlPO_4$
Difficulty available fixed phosphorus . . . . $FePO_4$, $Al_2(OH)_3PO_4$,
$Fe_2(OH)_3PO_4$

The basis of this division is the solubility of these compounds in 0.002 N H_2SO_4. Fixation, then, on these terms would be a change from the quickly or readily available form to the slowly or difficultly available forms, or to forms very slightly soluble in 0.002 N H_2SO_4 solution buffered to pH 3.0. Several writers still prefer to designate as fixed that proportion of the soluble phosphate salt which is retained by the soil when the soil-water suspension is filtered. Assuredly this connotation of the term is of little practical significance, as the amount removed by weak acids is not beyond the reach of plants. In this paper an effort has been made to differentiate between that portion which, in accordance with our conventional understanding of the weak acids' duplication of the solvent action of root hairs, is not extracted by acid and that which is. Citric acid of 1 per cent strength was used. Extraction with this solvent, according to Craig (7), gives results which agree fairly well with those obtained by the use of 0.002 N H_2SO_4.

During experimental work it was found that ordinary filtration, even through Whatman no. 50 filter papers, was quite unsuitable, as filtrates with few exceptions were cloudy and unsuitable for colorimetric work. Alundum thimbles also were rejected, as the sorption was found to be too high. Sintered glass crucibles, of grade 4 porosity and of 30 ml. capacity were used. These were tightly packed to a depth of about 0.25 cm. with kieselguhr, tapped down with a flattened glass rod. Neither kieselguhr nor the sintered crucible causes any sorption. The sintered plate is made of glass with no binding material such as that in alundum. Kieselguhr is composed chiefly of silica, and ordi-

nary white kieselguhr contains no phosphorus. In these experiments acid-washed kieselguhr was used, though the ordinary white product was quite suitable. Filtration under vacuum is this manner was very rapid and effective, and the crucibles were easily cleaned.

If many determinations are to be made, ordinary shaking in the cold would be too tedious and time-consuming. It was found that by refluxing the soil and solution, fixation could be effected in a very short time. The soluble phosphate solution used in these experiments was di-ammonium phosphate.[3]

A standard solution in which 1 ml. contained 0.5 mgm. P_2O_5 was prepared. Before use this was diluted so that 1 ml. contained 0.04 mgm. P_2O_5, which is equivalent to a dressing of 800 pounds per acre foot of soil. Such a dressing is in accordance with agricultural practice, particularly in a region where phosphate fixation does not appear to be so serious a problem as it is, for example, in Hawaii. The phosphate solution of which 1 ml. contained 0.04 mgm. P_2O_5 was found to have a pH value of 7.56. Ayres, as mentioned, considered it necessary to bring the phosphate solution to a point just below neutrality (pH 6.8). The writer, however, noted that the ammonium phosphate solution could not be kept at any predetermined pH under the conditions of the present tests. After this solution had boiled 5 minutes its pH was found to have fallen from 7.56 to 7.12; after 15 minutes, to 7.00; after 38 minutes, to 6.55; after 1 hour, to 6.28; and when the final pH was taken, after $1\frac{1}{4}$ hours, it had fallen to 6.13. Obviously, then, if soil and solution were to be boiled, adjusting the pH value to any known figure would be of little value. As the technic devised by the writer requires boiling of the solution, no effort was made to adjust the pH. It was further found unnecessary to add any electrolyte, such as ammonium chloride, as recommended by Ayres, Davis, Hance, and others, thus dispensing with a very undesirable feature of these experiments.

Experimental. Ten grams of air-dried soil passed through a 1-mm. sieve is placed in a 250-ml. Erlenmeyer flask, and 100 ml. of di-ammonium phosphate solution (1 ml. = 0.04 mgm. P_2O_5) is added. The contents are refluxed for 30 minutes, and 25 ml. is poured off into a measuring cylinder. To the remaining solution 0.75 gm. citric acid is added, and the boiling is continued for an additional 30 minutes. At the end of this time the solution is cooled and filtered, the filtrate being reserved for the colorimetric determination of phosphorus. The 25 ml. previously removed is filtered under vacuum as described. Even with very clayey soils not more than 15 minutes should be needed to obtain about 20 ml. of clear filtrate. One milliliter of this filtrate is used for the colorimetric determination of phosphorus by the well-known ceruleo-molybdate reaction, 1 ml. of the molybdate solution and 6 drops of stannous chloride solution being used. The phosphorus in 5 ml. of the citric acid extract

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[3] The test for the ammonia-phosphate ratio of this salt is given by the Committee on Analytical Reagents of the American Chemical Society (5). This test was confirmed and found accurate to 0.4 per cent by Ball (2).

TABLE 1

Fixation of phosphate in 12 soil samples

(On an acre basis)

SOIL SAMPLE	DESCRIPTION OF THE SOIL	PERIOD OF BOILING	P_2O_5 FIXED	P_2O_5 PERMA-NENTLY FIXED[*]	P_2O_5 PERMA-NENTLY FIXABLE
		minutes	*pounds*	*pounds*	*per cent*
1a	Gray-brown sandy loam (0–6 inch layer)	15	400	240	30
		30	500	240	30
		45	520	240	30
		60	540	240	30
1b	Dark gray-brown loam (7–12 inch layer)	15	468	410	51
		30	468	410	51
		45	487	400	50
		60	500	398	50
1c	Reddish yellow gravel containing 68 per cent Fe_2O_3 and 7.5 per cent Al_2O_3 (13–16 inch layer)	15	202	268	33
		30	202	240	30
		45	202	242	30
		60	288	268	33
1d	Yellow-gray silty clay (17–28 inch layer)	30	472	560	70
		60	472	512	64
1e	Yellow-gray fine sandy silt, weathered shale (29–40 inch layer)	30	400	552	69
		60	400	564	70
2	Dark brown or black clay loam	15	680	502	63
		30	688	502	63
		45	688	502	63
		60	720	502	63
3	Dark reddish brown clay loam	30	700	700	87
		60	720	700	87
4	Reddish brown sand	30	330	366	46
		60	366	366	46
5	Yellow-gray-brown sand	30	184	214	27
		60	184	214	27
6	Gray sand	30	720	266	33
		60	740	266	33
7	Reddish brown clay (laterite)	30	736	756	94
		60	740	756	94
8	Gray light sandy loam	30	244	230	29
		60	294	260	32

[*] Remaining fixed after boiling for an additional half hour with 1 per cent citric acid.

to which a little 10 per cent calcium acetate solution had been added was determined, after ignition, by extracting the residue with 5 ml. 10 per cent H_2SO_4 and making the extract up to 100 ml.   Twenty milliliters of this extract was treated in a similar manner as previously outlined.

In interpreting these results, that part of the fixed phosphate which could not be removed by citric acid treatment was regarded as permanent, in the sense that it was unavailable, or perhaps very slowly available, to plants. That part removed by citric acid was regarded as temporarily fixed.   Temporary fixation, then, need not be regarded as fixation from an agricultural point of view.

Table 1 shows the results obtained with 12 samples.   Table 2 gives the physical composition of the soils.   The eight soils actually used were selected from various points on the Natal coast and may be regarded as representative of fairly distinct types.

TABLE 2

*Physical composition of the soils**

SOIL SAMPLE	COARSE SAND	FINE SAND	SILT	CLAY	MOISTURE	DISSOLVED MATERIAL	DIFFERENCE†
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1a	13.6	53.4	12.4	15 3	2.7	0.6	2.0
1b	12.4	44 0	12 0	23 3	5.5	0 4	2.4
1c	82.2	4 7	2.3	7 0	4.1	0.2	0
1d	2.1	45.8	22 7	21.3	7.0	0.2	0.9
1e	1.0	62.8	19.8	11 4	4 1	0.5	0 4
2	12 2	27 2	16.1	37 1	7.1	1.0	0
3	14 2	29.3	10.0	39 2	8 2	0.6	0
4	56.5	26 4	3.6	10.4	1 7	0.1	1.3
5	67.3	25 7	2 4	2 8	0.2	0.2	1.4
6	61 9	27.5	1 6	4.3	0.4	2.4	1.9
7	4.3	11.8	15 9	55 8	8.3	0 7	3.2
8	48 6	34 8	6.8	7 0	0.7	0.4	1.7

* Mechanical analyses made by the Robinson pipette method.
† Including organic matter.

DISCUSSION OF RESULTS

Five profiles of sample 1 were examined, as this field, despite heavy dressings of phosphatic fertilizer (1,200 pounds an acre), has given no response whatsoever under sugar cane for a number of years.   Below 12 inches no phosphates have redissolved, indicating that fixation is taking place in a permanent form. It is intended subsequently to determine the correlation between the phosphate concentration in the different layers and the fixing power of the soil as determined by these experiments.

No attempt has yet been made to study the response of crops on the other soils used.   Sample 6 was from a field which had been heavily dressed with filter cake from a sugar factory and contained 1.84 per cent CaO soluble in HCl (1.115 s.g.).   This soil, when treated with a dressing of 10,000 pounds

P_2O_5 per acre foot, was found to fix 2,860 pounds P_2O_5 in the prescribed period of boiling.

It will be seen from table 1 that no great advantage is to be obtained by boiling longer than 30 minutes. It was also found in all the soils tested that no further phosphate was removed by continuing the citric acid treatment for 1 hour. It must be mentioned that the quantity of P_2O_5 remaining fixed after boiling with 1 per cent citric acid for an additional half hour is obtained by allowing for the amount removed normally by boiling with 1 per cent citric acid for half an hour. This separate determination, of course, must be made. Actually it will be found that the figures in column 5 are obtained by determining the percentage of fixation after adding together the tons of P_2O_5 still fixed and the amount dissolved by the straight treatment. Very conflicting results will be obtained unless the amount removed normally by 1 per cent

TABLE 3

Phosphate fixation in citric acid solution

SOIL SAMPLE	P_2O_5 PER ACRE FIXED IN PRESENCE OF 1 PER CENT CITRIC ACID*	SOIL REACTION	REACTION OF FINAL SOLUTION
	pounds	*pH*	*pH*
1a	374	5.30	2 23
1b	460	6 70	2 43
1c	300	8 06	2.37
1d	560	8 55	2 61
1e	528	8 57	2 61
2	528	8 42	2 82
3	664	6 37	2 58
4	288	4 90	2 24
5	254	6 39	2 23
6	120	8 60	3 45
7	740	6 11	2 66
8	166	5 44	2.32

* Period of boiling, one-half hour.

citric acid is accounted for. In sample 6 for example, the 1 per cent citric acid treatment after fixation revealed a content of 1,340 pounds P_2O_5 per acre. Of this, 800 pounds was found to be normally soluble in 1 per cent citric acid. Whether full allowance should be made for the phosphate normally dissolved by 1 per cent citric acid is uncertain. It is possible that the solution of the original phosphate in the soil may not normally be duplicated in the presence of new phosphate complexes formed in the process of boiling. No further citric acid was added to any of these soils to compensate for the effect of carbonates. Vanstone (38) found that field trials are usually in better agreement with the unmodified test. Despite many objections to the use of 1 per cent citric acid with calcareous soils (8, 25, 27) no alteration was made in the procedure. Soils as rich in calcium as is sample 6 are not common. Table 3 shows that the pH of the final solution was only 3.45. This indicates

a well-buffered solution, and as the main objective in these experiments was to effect fixation in an acid solution at constant pH for each test there seemed no reason to modify the solution used.   That in these experiments fixation occurred at pH 2 was due to the circumstance that 1 per cent citric acid, which was used because it is common in soil analysis, had this value.   Calcium, it must be remembered, is an ineffective agent below pH 6.5–6.7.   The purpose of choosing an acid solution was, in consequence, twofold: first, to prevent the formation of calcium phosphate, which is not so objectionable agriculturally as experimentally, and secondly, to approximate the acid conditions induced by the root hairs in proximity with the newly formed phosphate compounds of iron and aluminum.   Some investigators may consider pH 2 as too acid, but inasmuch as extensive fixation may occur at this low pH value the objections on such grounds are not really valid.   The phenomenon of sorption occurring at this or any other pH was not considered, as the rôle played by this phenomenon in retaining phosphates does not appear to be clearly defined.

No explanation can be offered for the fact that a number of these samples continue to fix a further quantity of phosphate in the presence of boiling 1 per cent citric acid.   The point was interesting and suggested that a direct boiling with di-ammonium phosphate in the presence of 1 per cent citric acid would in itself be a guide to the phosphate permanently fixable by the soil. This was done with each of the soils, and the results are given in table 3. These show a fair correlation, particularly with respect to the heavier soils. Though insufficient work has been done along these lines to warrant recommendation of the procedure of fixation of phosphates in the presence of 1 per cent citric acid, the method is promising.   The work of Wrenshall and McKibbin (39) and others leads us to believe that 1 per cent citric acid (pH 2.1) may be too drastic in its action and that perhaps some such well-buffered solution as acid calcium sulfate or acid potassium sulfate may be more suitable.   Such a consideration, however, is slightly beyond the scope of this paper.

PRACTICAL REASONS FOR THE STUDY

The purpose of the experiments described in this paper was to develop a practical method of defining the fixing power of a soil for soluble phosphate. If plants show no response to phosphatic fertilizer, it is evident that phosphorus has been fixed beyond the immediate reach of the plant.   That, as in soil 6, all the soluble phosphate should be fixed and yet a plentiful supply of available phosphate be at hand necessitated emphasizing in our definition only the permanent fixing power.

Several writers have remarked on the relation between various plant diseases and phosphate deficiency.   Heck (22) believes that phosphate deficiency has some relation to brown stripe, eye spot, and pythium root rot.   Although Hance (15) believes plants (D. 1135 sugar cane) can utilize tricalcium, ferric, aluminum, and reverted phosphates and hence that these compounds cannot be considered responsible for phosphate deficiency in plants, Scarseth and

Tidmore (35) are of the opinion that tricalcium phosphate (the least insoluble of these compounds) is not available to plants and decreases yield when precipitated. Truog (37) found that 9 out of 10 plants gave better growth on aluminum phosphate than on calcium phosphate and that 6 made better growth on ferric phosphate than on calcium phosphate. Heck (20) considers tricalcium phosphate readily available. It is evident from the foregoing conclusions that much work still remains to be done in this field. No attempt, however, is here made to solve these problems. The phosphate removed by 1 per cent citric acid must be considered, for all practical purposes, available to plants, and it has been established many times that applications of lime to high-fixing, moderately acid soils result in an increase of available phosphate. That the complex compounds formed in accordance with the physicochemical theory of fixation should be less available to plants than the simpler compounds formed by chemical decomposition seems highly probable.

Other methods suggested for overcoming this problem are the use of fertilizer briquettes (16, 17) and the judicious application of phosphatic fertilizer. Heck (22) recommends the application of a slow-fixing phosphate and at the same time stimulation, with crop residue, of the biological activity of the soil. Due consideration should also be given to the type of phosphatic fertilizer added. Snider (36), for example, studying phosphate response in soils found that, in general, the soils having the lowest relative solubility and the highest relative fixing power gave the greatest response to rock phosphate as measured by increase in crop yields. Scarseth and Tidmore (35) recommend the addition of phosphate in pellet form or in localized zones. The positional factor has received due emphasis by most workers. Phosphate penetration in high-fixing soils is impeded, hence phosphate should be applied with due consideration to the feeding zones of roots. Hockensmith, Gardner, and Kezer (24) found that the depth of applications of superphosphate markedly affects the yield of alfalfa. With applications at a depth of 4 inches the increase was 179.0 per cent in comparison with an increase of only 102.2 per cent with applications 1 inch below the surface. Superphosphate placed on the surface remains largely where it is unless a flooding method of irrigation is used. In view of the foregoing remarks it would appear that the most favorable application to sugar cane would be in drills between the rows.

SUMMARY

The object of this paper was to study our present knowledge of phosphate fixation and to describe a practical method for its determination in the laboratory.

The meaning of phosphate fixation is examined, and the effects of pH, exchangeable bases, organic matter, time, and soil composition on this phenomenon are briefly outlined.

The physicochemical theory of phosphate fixation is discussed.

Reference is made to the laboratory work of other writers.

A method is given for the determination of phosphate fixation. By the use of 1 per cent citric acid the permanently fixable phosphate was distinguished from that fixed only temporarily.

The experiments were repeated to examine fixation in the presence of 1 per cent citric acid at approximately pH 2.5. The amount of phosphate fixed in the presence of 1 per cent citric acid compared very favorably with the amount finally obtained by the first procedure, and the method suggests itself as an easy and rapid alternative for determining phosphate fixation.

The practical issues of the study are discussed.

REFERENCES

(1) AYRES, A. 1934 Phosphate fixation in Hawaiian soils: II. *Hawaii. Planters' Rec.* 38: 131–145.

(2) BALL, T. R. 1930 The acidity of mono and diammonium phosphates. *Jour. Amer. Chem. Soc.* 52: 3901–3905.

(3) BROWN, B. A., AND MUNSELL, R. I. 1933 Pasture investigations (second report). Penetration of surface applied lime and phosphates in the soil of permanent pastures. Conn. (Storrs) Agr. Exp. Sta. Bul. 186.

(4) BROWN, L. A. 1935 A study of phosphorus penetration and availability in soils. *Soil Sci.* 39: 277–287.

(5) COLLINS, W. D., ET AL. 1927 Recommended specifications for analytical reagent chemicals. *Indus. and Engin. Chem.* 19: 645–649.

(6) COOK, R. L. 1935 Divergent influence of degree of base saturation of soils on the availability of native, soluble, and rock phosphate. *Jour. Amer. Soc. Agron.* 27: 297–311.

(7) CRAIG, N. 1934 Some properties of the sugar cane soils of Mauritius. *Mauritius Dept. Agr. Sugarcone Res. Sta. Bul.* 4: 9.

(8) DAS, S. 1936 The determination of available phosphoric acid of calcareous soils. India Dept. Agr. Mem., Chem. Ser. 8, no. 6.

(9) DAVIS, L. E. 1935 Sorption of phosphates by non-calcareous Hawaiian soils. *Soil Sci.* 40: 129–158.

(10) DAVIS, L. E. 1935 Handbook of Hawaiian Soils, p. 78. Assoc. Hawaii. Sugar Tech., Honolulu, Hawaii.

(11) DEAN, L. A. 1934 Electrodialysis as a means of studying the nature of soil phosphates. *Soil Sci.* 37: 253–266.

(12) DOUGHTY, J. L. 1930 The fixation of phosphate by a peat soil. *Soil Sci.* 29: 23–35.

(13) DOUGHTY, J. L. 1935 Phosphate fixation in soils, particularly as influenced by organic matter. *Soil Sci.* 40: 191–202.

(14) FORD, M. C. 1933 The nature of phosphate fixation in soils. *Jour. Amer. Soc. Agron.* 25: 134–144.

(15) HANCE, F. E. 1933 Phosphate fixation in Hawaiian soils. *Hawaii. Planters' Rec.* 37: 182–196.

(16) HANCE, F. E. 1934 Plant feeding by briquette fertilization. *Soil Sci.* 37: 445–457.

(17) HANCE, F. E. 1935 The preparation of fertilizer briquettes and their utilisation in general agricultural practice. *Trans. Third Internatl. Cong. Soil Sci.* 1: 258–260.

(18) HANCE, F. E., AND YUEN, Q. H. 1935 Phosphate fixation in Hawaiian soils—IV. Rapid methods for determining capacity, rate, degree and differentiation of fixation. *Hawaii. Planters' Rec.* 39: 97–108.

(19) HECK, A. F. 1934 Phosphate fixation and penetration in soils. *Soil Sci.* 37: 343–355.

(20) HECK, A. F. 1934 A method for determining the capacity of a soil to fix phosphorus in difficultly available form. *Soil Sci.* 37: 477–482.

(21) HECK, A. F.   1934   Effect of the degree of base saturation of a soil on its capacity to fix phosphorus in difficultly available form. *Soil Sci.* 38: 463–470.

(22) HECK, A. F.   1935   Availability and fixation of phosphorus in Hawaiian soils. *Jour. Amer. Soc. Agron.* 27: 874–884.

(23) HIBBARD, P. L.   1935   Factors influencing phosphate fixation in soils. *Soil Sci.* 39: 337–358.

(24) HOCKENSMITH, R. D., GARDNER, R., AND KEZER, A.   1933   The effect of depth of placement on the availability of superphosphate in calcareous soils. *Soil Sci.* 36: 35–39.

(25) McGEORGE, W. T.   1934   The determination of phosphate availability in calcareous soils by electrodialysis. *Soil Sci.* 38: 347–353.

(26) McGEORGE, W. T.   1935   The relation of potential alkalinity to the availability of phosphate in calcareous soils. *Soil Sci.* 39: 443–452.

(27) McGEORGE, W. T., AND BREAZEALE, J. F.   1931   Phosphate solubility studies on some unproductive calcareous soils. Ariz. Agr. Exp. Sta. Tech. Bul. 35.

(28) McGEORGE, W. T., AND BREAZEALE, J. F.   1932   Studies on iron, aluminum and organic phosphates, and phosphate fixation in calcareous soils. Ariz. Agr. Exp. Sta. Tech. Bul. 40.

(29) McGEORGE, W. T., BUEHRER, T. F., AND BREAZEALE, J. F.   1935   Phosphate availability in calcareous soils: A function of carbon dioxide and pH. *Jour. Amer. Soc. Agron.* 27: 330–335.

(30) MATTSON, S.   1931   The laws of soil colloidal behavior: VI. Amphoteric behavior. *Soil Sci.* 32: 343–365.

(31) PUGH, A. J.   1934   Laws of soil colloidal behavior: III. Colloidal phosphates. *Soil Sci.* 38: 315–334.

(32) RAVIKOVITCH, S.   1934   Anion exchange: I. Adsorption of the phosphoric acid ions by soils. *Soil Sci.* 38: 219–239.

(33) SCARSETH, G. D.   1935   The mechanism of phosphate retention by natural aluminosilicate colloids. *Jour. Amer. Soc. Agron.* 27: 596–616.

(34) SCARSETH, G. D., AND TIDMORE, J. W.   1934   The fixation of phosphates by soil colloids. *Jour. Amer. Soc. Agron.* 26: 138–151.

(35) SCARSETH, G. D., AND TIDMORE, J. W.   1934   The fixation of phosphates by clay soils. *Jour. Amer. Soc. Agron.* 26: 152–162.

(36) SNIDER, H. J.   1934   The solubility of phosphorus in soils from some Illinois experiment fields. *Soil Sci.* 38: 471–476.

(37) TRUOG, E.   1916   The utilization of phosphates by agricultural crops including a new theory regarding the feeding power by plants.   Wis. Agr. Exp. Sta. Res. Bul. 41.

(38) VANSTONE, E.   1925   The available phosphate in soils. *Jour. Agr. Sci.* 15: 460–465.

(39) WRENSHALL, C. L., AND McKIBBIN, R. R.   1935   A comparison of some methods used in extracting soil phosphates, with a proposed new method. *Jour. Amer. Soc. Agron.* 27: 511–518.

FRACTIONAL APPLICATION OF NITROGENOUS FERTILIZERS ON SPRING WHEAT UNDER IRRIGATION[1]

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Experiments on the fractional application of nitrogenous fertilizers on spring wheat under irrigation were conducted at the Ershov Agricultural Experiment Station (Transvolga region, Saratov Province). The dark chestnut-brown soils of this region are fairly rich in nutrients, but because of the low rainfall (about 300 mm. a year), the crops without irrigation give low yields. The average yield of spring wheat on these soils without irrigation and without fertilizer is about 10 centners[2] per hectare.

The basis for the experiments on the fractional application of nitrogenous fertilizers was preliminary work on the problem of the utilization of soil nutrients by spring wheat. That work showed that during the growth period, wheat does not use soil nitrogen uniformly. The period of maximum utilization of nitrogen coincides with the rapid development of the stem and the heading out of the wheat, from tillering to the beginning of flowering. Wheat also uses large quantities of nitrogen when the kernels fill out, and if nitrogen is lacking at that period, the protein content of the wheat is lowered. Gerrick, Davidson, and a number of Russian investigators who worked with fertilizers on spring wheat formulated a series of valuable postulates, which were utilized in field experiments.

EXPERIMENTS IN 1934

In 1934, 80 kgm. of nitrogen as ammonium sulfate and 90 kgm. of P_2O_5 as superphosphate were used per hectare. The superphosphate was broadcast and harrowed in before spring planting. At the same time, 50 kgm. of nitrogen was applied. The rest of the ammonium sulfate was applied in equal portions, one portion of which was broadcast before the first irrigation, which naturally washed it into the soil, and the other was introduced with the second irrigation, the sulfate being dissolved in the irrigation water. Thus, with two irrigations, the nitrogen fertilizer was introduced in three fractions—at the time of planting, during tillering, and at the time of heading out.

Detailed investigations were made on the dynamics of nitrates and ammonia

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[1] Translated from the Russian manuscript by J. S. Joffe, New Jersey Agricultural Experiment Station.

[2] One centner equivalent to approximately 100 kgm.

in soils when the fertilizers were applied at various times. The results, reported in table 1, show very definitely that when all the nitrogen was applied before planting, the quantity of nitrates in the soil decreased very markedly during the growth period of the wheat and at the stage of grain formation the soil apparently lacked nitrogen for the further growth of the wheat. This decrease is due to the intensive utilization of nitrogen by the wheat plants before heading out. When fractional applications of nitrogenous fertilizers were made, however, the supply of nitrate nitrogen was constant throughout the normal period of development of the wheat. It is important to note that in the fractional applications the quantity of nitrate nitrogen was uniformly distributed throughout the entire 50-cm. layer of the soil; whereas in the single

TABLE 1

Effect of time of application of nitrogen on nitrate content of 0–50 cm. layer of soil

DATE OF NO₃ DETERMINATION	NO₃ FOUND PER KILOGRAM OF SOIL	
	Nitrogen applied before planting	Nitrogen applied before planting and at time of heading
	mgm.	*mgm.*
May 25.......	56.9	26.7
June 2........	19 5	23.3
July 2.......................................	9 6	21.7

TABLE 2

Morphological analysis of the wheat samples, 1934

	NITROGEN APPLIED BEFORE PLANTING	NITROGEN APPLIED BEFORE PLANTING AND AT HEADING OUT	NITROGEN APPLIED AT 1ST AND 2ND IRRIGATIONS
Energy of tillering.................	3.3	3.6	3.5
Energy of heading out	2 8	3 5	2.9
Height of plant..................... .....*cm.*	108	117	112

application of nitrogen the nitrates were distributed only in the upper surface of the layer.

The fractional application of nitrogenous fertilizers altered considerably the process of development of the wheat. Before the first irrigation, the wheat on the plot that received the nitrogen in one application developed considerably better than the wheat on the plot to which the fractional application was made; but after the first irrigation the wheat on the plot to which fractional application was made began to develop rapidly, and eventually its growth exceeded that of the wheat on the first plot (table 2).

Chemical analyses of samples of wheat taken from the plots under various conditions of fertilization showed definitely that under irrigation the fractional applications of nitrogen allowed a higher accumulation of nitrogen in

the plants, thereby producing better plants than those produced with the single application of nitrogen. Table 3 shows the ash, nitrogen, and phosphorus content of wheat grown with a single application of nitrogen as compared with that of wheat grown with fractional applications at planting and at heading out.

The yield of spring wheat on these experimental plots is shown in table 4. The following conclusions may be drawn from the data presented: Fractional applications of nitrogenous fertilizers during the various phases of growth of wheat give a higher yield than do the ordinary methods of applying the fertilizer before planting. The highest yields of grain were obtained when 50 kgm. of nitrogen was applied before planting and 30 kgm. at the time of heading and

TABLE 3

Ash, nitrogen, and phosphorus in the tops of wheat (gm. per 50 plants)

DATE OF ANALYSIS	NITROGEN ADDED BEFORE PLANTING			NITROGEN ADDED AT PLANTING AND AT HEADING OUT		
	Ash	N	P	Ash	N	P
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
May 26.. . . .	1 27	0 32	0 066	0 99	0 23	0 047
June 28..	31 64	5 07	0 339	31 41	5 29	0.332
August 11*..... . .	27 19	6 47	2 189	35 27	8 26	2 514

* At maturity.

TABLE 4

Yield of spring wheat with single and fractional applications of nitrogen, 1934

NITROGEN APPLIED	YIELD OF GRAIN PER HECTARE	WEIGHT OF 1,000 KERNELS	PROTEIN CONTENT
	centners	*gm.*	*per cent*
Before planting.	26 8	35 3	17 13
Before planting and at heading out	29 7	36 8	18 69
At first and second irrigations..	29 9	35 7	18 35

when the fertilizers were introduced at the first and second irrigations. The increase in yield as a result of fractional application of nitrogen was about 3 centners per hectare, or 12 per cent. In other words, simply modifying the time of applying the nitrogenous fertilizers increased their effectiveness, in terms of yields, by 12 per cent.

EXPERIMENTS IN 1935

In 1935, the experiments on the fractional application of nitrogen for spring wheat were repeated on the Ershov irrigation experimental plot. The plan of the experiments was similar to that in 1934. Ninety kilograms of P_2O_5 in the form of superphosphate was added per hectare in the fall when the land was plowed. One plot received 90 kgm. of nitrogen in the spring before plant-

ing. A second plot received 30 kgm. of nitrogen before planting and an additional 60 kgm. before the second irrigation. A third plot received 30 kgm. of nitrogen before planting, 30 kgm. before the first irrigation, and 30 kgm. with the second irrigation. Just as in 1934, the plot to which the nitrogen was added in one application showed a better development of the plants in the early stages. During tubulation, 48 gm. of dry material per hundred plants accumulated. When the nitrogen application was split, the accumulation of dry matter at this point was only 43 gm. Later, the picture changed, and the fractional application of nitrogen resulted in a better development of the plants and a higher accumulation of dry matter. In table 5 the accumulation of the dry matter in 100 plants under various conditions is given.

TABLE 5

Accumulation of dry matter under various conditions of nitrogen application (gm. per 100 plants)

NITROGEN APPLIED	DRY MATTER ACCUMULATED			
	Beginning of tubulation	Beginning of heading out	Filling out of grain	Complete maturity
	gm.	*gm.*	*gm.*	*gm.*
Before planting.........	48 4	202 4	350 6	427.0
Before planting and at second irrigation.... ..	44 1	230.6	407 5	583.0
Before planting and at first and second irrigations..	43 3	199 1	394.8	525.0

TABLE 6

Yield of spring wheat with single and fractional applications of nitrogen, 1935

NITROGEN APPLIED	YIELD OF GRAIN PER HECTARE	WEIGHT OF 1,000 KERNELS
	centners	*gm.*
No fertilizer, with two irrigations.....................	19.7	40.7
Before planting...........	24 2	41 3
Before planting and at second irrigation	26 9	40 7
Before planting and at first and second irrigations.....	25.0	38.1

The tillering and the heading out of the wheat also were similar to those observed in 1934. At the end of May, the tillering index was 3.1 for the wheat on the plot to which the nitrogen was added in one application and 2.74 for the wheat on the plots receiving the fractional application of nitrogen. Gradually this relation was reversed: on June 26 the thickness of the tillering on the plot with the single application of nitrogen had an index of 1.62, whereas the plots with the fractional application of nitrogen had an index of 2.15; at maturity the index on the first plot was 1.90, and that on the others was 2.31.

The data on yields for 1935 are given in table 6. As in 1934, the yield with the fractional application of ammonium sulfate was appreciably higher than that with the single application. In 1935, the increase was 2.7 centners of

grain for the fractional application in two periods and slightly less for the fractional application in three periods.   The lowering of the yield in the latter case may be explained by the late development of the plants, which thus were subjected somewhat to the effects of drought.

The observations on the nitrates in the soil showed results similar to those in 1934.   With the single application of fertilizer, the nitrates were high in the beginning of the growth period, were decreased very markedly at the milk stage, and were reduced to mere traces at maturity.   Thus, a single application of the fertilizer does not supply the plants with nitrogen uniformly throughout the growth period.   The fractional application of nitrogen, on the other hand, provides a uniform supply of nitrogen throughout the growing season.   As in the 1934 experiment, the grain of the wheat grown on the plots to which the nitrogen was added fractionally had a higher content of protein than the grain of the plots which received a single application of nitrogen.   In 1935, it was most convenient to introduce the nitrogen fertilizers at two intervals, before planting and with the second irrigation.   These resulted in the best yield and quality of grain.

EXPERIMENTS AND OBSERVATIONS IN 1936

In 1936, which was distinguished by an especially dry summer, the experiments with the fractional application of nitrogenous fertilizer were repeated and, as in 1935, 90 kgm. of P and 90 kgm. of N were added.   On the second and third plots, the fractional applications were made as in previous years.   Because of the extremely dry weather of 1936, however, the first irrigation was not given early enough.   The absolute yield of wheat was considerably lowered therefore, but the tendencies which were apparent in 1934 and 1935 were again evident.   With the phosphorus and nitrogen, potash was also added, and one could trace the influence of fractional application of nitrogenous fertilizers with the potash and phosphorus as the basic fertilizer.

As the progress of the growth of the spring wheat is similar to that in the previous experiments, the yield data alone are given.   Where 90 kgm. of P and 90 kgm. of N were added before planting, the yield of grain was 12.4 centners per hectare.   Where 90 kgm. of P and 60 kgm. of N were added before the first irrigation, and 30 kgm. of N before the second irrigation, the yield of the grain was 15.1 centners per hectare.   Where 90 kgm. of P, 60 kgm. of N, and 45 kgm. of K were added before the first irrigation, and 30 kgm. of N before the second irrigation, the yield was 15 centners of grain per hectare. Thus, the addition of the nitrogen fertilizer at various intervals, as compared with the single application, gave an increase of 2.4 to 2.6 centners of grain per hectare.

In 1936, Nikolaev, a member of the staff of the All-Union Institute of Grain Culture at Saratov, conducted an experiment on the fractional application of fertilizers, with special reference to nitrogen, at the Chapaev collective (kolkhoz) in the Saratov region.   The spring wheat Melyanopus 069 was used,

and five irrigations were given during the growth period. The first irrigation was at the beginning of tillering; the second, during tubulation; the third, during heading out; the fourth, at the beginning of flowering; and the fifth, at the time of filling out of the grain. The mineral fertilizers added per hectare were as follows: 100 kgm. of P in the form of superphosphate, 120 kgm. of N in the form of ammonium sulfate, and 45 kgm. of K in the form of sylvinite. All of the superphosphate was added at the time of planting and was cultivated into the soil. Thirty kilograms of nitrogen per hectare was broadcast before the first irrigation, and 30 kgm. per hectare was dissolved and added with each of the second, third, and fourth irrigations. The sylvinite was dissolved and added with the fifth irrigation.

The growth period of the Melyanopus wheat, with one irrigation and without fertilizer, was 81 days; with three irrigations, without fertilizers, 85 days; with four irrigations and fertilizers, 97 days; and with five irrigations and a complete fertilizer, 102 days. Thus the growth period of the wheat ranged from 81 to 102 days.

The paucity of water and nutrients in the soil decreased not only the period of vegetation but also the accumulation of dry matter. Thus the wheat with one irrigation and without fertilizer yielded 10.25 centners of dry matter per hectare; with nitrogen and phosphorus and two irrigations, 20.2 centners; with the complete fertilizer and five irrigations, 162.2 centners.

Under actual production conditions, on an area of 3.5 hectares, with 100 kgm. P, four irrigations, and fractional application of 120 kgm. of N, the yield was 131.8 centners per hectare, of which 45.8 was grain and 80 straw. On an area of 1.4 hectares, with five irrigations and a fractional application of 120 kgm. of N in addition to the 100 kgm. of P and 45 kgm. of potassium, the yield of grain was 57.42 centners per hectare and 105.41 centners of straw. The absolute weight of 1,000 kernels from the 3.5-hectare area was 36 gm.; from the 1.4-hectare area, 36.6 gm. Thus, under actual production conditions, a substantiation of the experimental data was obtained.

SUMMARY

Experiments were conducted for 3 years and observations were made under actual production conditions on the effect of fractional applications of nitrogenous fertilizers during the growth period of spring wheat under irrigation as compared with a single application before planting. The results demonstrate that when the supply of moisture is adequate the wheat utilizes the fractional applications more efficiently than the single application because with the fractional applications the plants obtain the necessary quantity of nitrogen throughout the period of their development. As a result, a higher yield is obtained and the quality of the crop is improved, as measured by the greater absolute weight and the higher protein content of the kernels.

ANION ADSORPTION BY SOIL COLLOIDS IN RELATION TO CHANGES IN FREE IRON OXIDES[1]

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The soil colloidal systems are heterogeneous in nature, but in general they may be classified as weathered fragments of the original soil minerals and as new formations. These new formations exist as more or less dehydrated gels inclosing the original mineral particles (2). The gels do not obey the law of definite chemical composition but should be pictured as complexes of variable composition (7) altering in structure and behavior with the ions in the soil solution.

The gels may be the hydrated oxides of iron, aluminum, or silica. The first two oxides do not possess any appreciable cation exchange powers but do retain certain anions, especially the phosphate and silicate (7), over a wide range of hydrogen-ion concentrations. The adsorption of anions by the oxide gels, which can function as acidoids, imparts to the gels new properties. Mattson (9) has elucidated the relation between the adsorption of silicates and phosphates and the increase in the cation exchange capacity in soil colloids. This phenomenon was observed by Prince and Toth (10) to be true in soils when phosphates and organic matter were added.

Mineralogical investigations of soil colloidal clays indicate that the free oxides of iron generally exist in them (12). As it has been shown by Ford (5) that the hydrated oxides of iron adsorb phosphates and by Mattson (9) that the retention of this anion by soil colloids increases the exchange capacity, reductions in free iron oxides might be expected when soil colloids adsorb phosphates.

With the latter point as a basis, the following experiments were initiated to study the reductions in the free iron oxide content in two highly ferruginous colloids produced as the result of phosphate and silicate ion adsorption. Simultaneously attempts were made to study the mechanism of phosphate adsorption by the colloids and changes in ultimate pH and cation exchange capacity.

Colloids were extracted from the following soil types:

Colts Neck loam: The B-horizon of a virgin profile approximately 1½ miles southeast of Red Bank, N. J.

Sassafras loam: The B-horizon of a virgin profile on the Wolpert Farm of the New Jersey Agricultural Experiment Station, New Brunswick.

Cecil clay subsoil: The 10–28 inch depth of a profile collected near Statesville, N. C.

Sharkey clay subsoil: Sample obtained near New Orleans, La.

EXPERIMENTAL METHOD

The colloids used in the studies that follow were isolated from four widely different soil types by agitating the soils with distilled water containing sufficient ammonia to render the dispersion alkaline and allowing the suspensions to settle for 18 hours. The coarser fractions from the portions of the suspension that were siphoned off were removed by centrifuging the sol in a low speed centrifuge (1200 r.p.m.) for 15 minutes. The centrifuged sol was then passed through a Sharples supercentrifuge at 22,000 r.p.m., which completely removed the dispersed particles. The colloids in the moist condition after removal from the centrifuge were placed in a Mattson cell (6) and dialyzed free of exchangeable cations. The H-clays thus obtained were dried in an electric oven at 30°C. and ground in an agate mortar to pass a 60-mesh sieve.

To obtain maximum saturation of the colloids with phosphates and silicates, the adsorption experiments were conducted in concentrated solutions and at decreasing pH values. This was done to prevent mobilization of certain acidic groups that occur at high pH values (10) and to increase the retention of the anions, as it has been shown (1, 7) that anion retention increases with decreasing pH values. With the phosphated and silicated series, duplicate 7-gm. portions of the respective colloids were suspended either in 200 cc. of ammonium phosphate and phosphoric acid mixtures or in solutions of sodium silicate to which sufficient dilute hydrochloric acid had been added to obtain variations in pH values. The anion concentrations ranged from 10 to 50 m.e. per 200 cc. of the solutions. After adsorption had proceeded for 2 days, aliquots of the clear supernatant liquid were withdrawn for P_2O_5 or SiO_2 analysis and pH determinations. The suspensions were then transferred to filter paper, allowed to drain, and to air-dry. The dried samples were reground to pass a 60-mesh sieve, and the various analytical procedures were made on duplicate samples.

To obtain H-clays, duplicate 4-gm. samples of the clays after anion adsorption were electrodialyzed for 12 hours in a Mattson cell. This period of electrodialysis was sufficient to render the colloids H-saturated.

The chemical composition of the original H-colloids was determined by following the A.O.A.C. methods outlined for the analysis of soils. The colloids were also examined for the contents of the free oxides of Fe, Al, and Si by the methods of Drosdoff and Truog (4); the electrodialyzed colloids were also analyzed for the content of free iron oxide by the latter method.

The cation exchange capacity of the phosphated and silicated colloids, both before and after electrodialysis, was determined by the barium acetate method outlined elsewhere (10).

The ultimate pH values of the untreated clays and of the clays which had adsorbed anions were determined on the electrodialyzed samples. The pH values were determined colorimetrically on the clear supernatant liquid of 1:10 suspensions after the colloidal particles had settled.

The electrodiffusible phosphates in the anode dialyzates from the phosphated samples and the quantities of phosphates mobilized by the preliminary sodium carbonate treatment in the Truog and Drosdoff method (4) were determined by the standard volumetric method.

CHEMICAL COMPOSITION OF THE ISOLATED COLLOIDS

The chemical composition and certain physicochemical properties of the isolated colloids are presented in table 1. Extreme variations in the individual chemical constituents and in the silica-sesquioxide ratios of the different soil colloids are apparent. A minimum silica-sesquioxide ratio of 0.81 occurs with the Colts Neck colloid; and a maximum ratio of 3.37, with the Sassafras.

TABLE 1

Chemical composition and certain properties of the isolated colloids

COLLOID	$\frac{SiO_2}{R_2O_3}$	SiO₂	Fe₂O₃	Al₂O₃	P₂O₅	CaO	MgO	C	H₂O (105°C.)	LOSS ON IGNITION	ULTIMATE pH	CATION EXCHANGE CAPACITY
		per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent		m.e./ gm.
Colts Neck...	0 81	27 47	36 03	32 71	1 10	0 09	1 00	1 24	5 27	10 03	4 90	0 319
Cecil..	1.01	34.48	14 34	47 12	0 05	0 04	0 58	0 48	2 23	4 09	5 30	0.107
Sharkey..... .	2 72	54 67	6.63	30 05	0.11	0 11	2 64	1 26	5 69	9.13	4 05	0.650
Sassafras......	3 37	53 02	15.69	16 65	0 36	0 36	0.79	0 79	3.25	6 49	5 10	0.206

The ratios for the Cecil and the Sharkey very nearly approximate the reported ratios of other investigators for these colloids (1, 6). The very marked difference between the average reported ratio of 1.85 (1, 6) and the value of 3.37 recorded for the Sassafras may be due to the presence of free quartz in the isolated clay.

A general relation between the composition of soil colloids and certain properties, especially the exchange capacity, has been elucidated by Anderson and Mattson (1). Colloids with a low silica-sesquioxide ratio possess lower exchange capacity than those of a high ratio. Mattson (7, 8) in his later investigations pointed out that since phosphates and humates, as well as silica, behave as acidoids, the relation between the exchange capacity and the composition should be evaluated in terms of the "active" acidoid-basoid ratio rather than in terms of the silica-sesquioxide ratio. From table 1 it can be seen that no correlation exists between the latter ratio of the colloids and the exchange capacity, the reason being that the isolated clays contain appreciable

quantities of organic matter and phosphorous.   As it is impossible to calculate the "active" acidoid-basoid ratio of any colloid because of the complexity of its makeup, an expression of the strength and the quantity of the acidic residues in soil colloids may be utilized to express variations in that ratio.   The pH of distilled water suspensions of H-clays yields the so-called "ultimate pH," which does express to some extent variations in the "active" acidoid-basoid ratio.   Colloids with decreasing ultimate pH values possess increasing cation exchange capacities; however, it is impossible to predict from these values the absolute magnitude of the cation exchange capacity.   If the colloids enumerated in table 1 are compared on the basis of increasing acidic strength and exchange powers, the clays can be arranged in the following order: Cecil, Sassafras, Colts Neck, and Sharkey.

The colloids were also analyzed for the contents of free SiO_2, Al_2O_3, and Fe_2O_3, and the results are recorded in table 2.   The colloids are very low in amorphous SiO_2 and free Al_2O_3, but the values for the free Fe_2O_3 are consid-

TABLE 2

Analysis of colloid by the Drosdoff and Truog method (4)

COLLOID	AMOR-PHOUS SiO_2	FREE Al_2O_3	FREE Fe_2O_3	P_2O_5 MOBILIZED	ORGANIC MATTER MOBILIZED	CATION EXCHANGE CAPACITY	
						Original colloid	Colloid minus free Fe_2O_3
	per cent	*per cent*	*per cent*	*mgm./gm.*	*mgm./gm.*	*m.e./gm.*	*m.e./gm.*
Colts Neck	0 29	0.66	24 80	3.00	2 0	0 319	0 260
Cecil.................	0 02	0.52	12 32	trace	None	0 107	0.110
Sharkey......	0.37	0.14	2.72	trace	52.0	0.650	0.437
Sassafras..............	0 20	0 62	7 58	trace	None	0 206	0.224

erably higher and show tremendous variations.   The exchange capacity of the mineral residues after the removal of the free iron oxides is also presented in this table.   Although Drosdoff and Truog (4) data indicate very little change in the exchange capacity of colloidal clays after the removal of the free oxides, the data for the Colts Neck and Sharkey colloids are not in line with the findings of these authors.   It is possible that the soil colloids examined by Drosdoff and Truog did not contain appreciable quantities of organic matter and phosphorus.   The reduction in exchange capacity is the result of mobilization of acidic groups (humus from Sharkey and phosphates from the Colts Neck) from the colloids that possess exchange powers.

ADSORPTION OF PHOSPHATES BY DEFERRATED CECIL AND SASSAFRAS COLLOIDS FROM PHOSPHORIC ACID SOLUTIONS IN RELATION TO CHANGES IN EXCHANGE CAPACITY

The removal of the free iron oxides from colloids produces very marked changes in the ability of the clays to retain the phosphate anion.   Data are

presented in table 3 on the adsorption of phosphoric acid ions by two colloids containing the oxides, and these adsorption data are compared with the retention by the same colloids after the removal of the oxides. The Cecil and the Sassafras colloids were chosen for this experiment as only slight changes were observed in the cation exchange capacities of the clays after the removal of the free oxides.

The removal of the free oxides from the Cecil colloid (12.32 per cent free iron oxides) reduced the adsorption of P_2O_5 from 0.370 to 0.205 m.e. per gram, whereas the reduction with the Sassafras was from 0.275 to 0.125 m.e. per gram. The lower adsorption by the mineral residues is also reflected in the exchange capacities of the phosphated clays. The Cecil colloid containing the free oxides, after phosphation, had an exchange capacity of 0.205 m.e., whereas the phosphated residue had exchange powers equivalent to only 0.137 m.e. per gram. A similar behavior is to be noted with the Sassafras colloid.

TABLE 3

Adsorption of phosphoric acid ions by Cecil a nd Sassafras colloids with and without the free iron oxides

COLLOID	P_2O_5 AS H_3PO_4	P_2O_5 ADSORBED	CATION EXCHANGE CAPACITY	INCREASE IN EXCHANGE CAPACITY
	m.e. in 50 cc.	*m.e./gm.*	*m e./gm.*	*m.e./gm.*
Cecil			0.107	. . .
Cecil.	2 00	0 370	0 205	0 098
Cecil with oxides removed		.	0 110	
Cecil with oxides removed	2 00	0 205	0 137	0 027
Sassafras.			0 206	.
Sassafras.	2 00	0 275	0 364	0.162
Sassafras with oxides removed	. .	.	0 224	. . .
Sassafras with oxides removed	2 00	0 125	0.246	0.022

It must be admitted that the total adsorptive surface may have been altered by the solution of certain micellae during the treatment of the clay; however, since the exchange capacities of the deferrated clays are approximately the same as those of the original colloids, this point may not be of very great importance. The lower values for the adsorption of phosphates and increases in the cation exchange capacity of the phosphated clays may be due solely to the removal of the free oxides. These oxides, upon the adsorption of phosphates, behave as new stable exchange groups.

ADSORPTION OF PHOSPHATES AND SILICATES BY THE VARIOUS COLLOIDS AND CHANGES IN CERTAIN COLLOIDAL PROPERTIES

The complete data on the adsorption of phosphates by the four colloids, on the changes in exchange capacity, and on the reductions in ultimate pH are presented in tables 4 and 5. The values of displaced SiO_2 from the various colloidal systems are also given in table 4.

Certain definite conclusions can be drawn from the data in table 4. Phosphate adsorption by the various colloids generally increases with decreasing silica-sesquioxide ratio and increasing content of free iron oxides. This relation holds rather well for the Cecil, Colts Neck, and Sassafras colloids, but the Sharkey colloid, with an oxide content of only 2.72 per cent, adsorbs approximately the same amount of phosphates as do the Cecil and Sassafras. With the Cecil and Sassafras colloids the free iron oxide content is about six times as great and three times as great, respectively, as that of the Sharkey colloid.

TABLE 4

Adsorption of P_2O_5 by soil colloids at different pH values in relation to the $\dfrac{SiO_2}{R_2O_3}$ ratio and free iron oxides content of the colloids

COLLOID	$\dfrac{SiO_2}{R_2O_3}$	FREE IRON OXIDES	P_2O_5 ADDED AS H_2PO_4 PLUS $(NH_4)_2HPO_4$	pH OF SUPERNATANT LIQUID AFTER ADSORPTION	P_2O_5 ADSORBED	DISPLACED SiO_2
		per cent	*m.e./200 cc.*		*m.e./7 gm.*	*m.e./7 gm.*
Colts Neck	0 81	24 80	10 0	6 71	2.67	. . . . .
			20.0	5 32	3.99	8.76
			40 0	4 98	5 68	10 62
			50 0	4 05	7 22	13.79
Cecil	1 01	12.32	10.0	6 87	1.05	. . . . .
			20.0	5.79	2.60	3.31
			40 0	5.11	3 50	5 96
			50 0	4 04	4.17	7.83
Sharkey	2 72	2.72	10.0	6.20	2.64	. . . . .
			20.0	4 52	3.70	11 56
			40.0	3 50	4.61	13.42
			50 0	3.30	5.14	14 54
Sassafras	3.37	7.78	10.0	6.70	1 60	. . . . .
			20.0	5 15	2.50	5.50
			40.0	4 70	3 46	8.19
			50.0	3.76	4.41	11.56

At lower pH values the four colloids adsorb more phosphates than at higher pH values.

The high adsorption value for the Sharkey colloid is probably due to a replacement of silicate ions from the surface layer of the lattice of this clay by the phosphate ion. This result is in line with the findings of Mattson (7) with this colloid. It is to be noted that a displacement of silica by the phosphate ion occurs with all the colloids, but that with the Sharkey colloid the replacement is nearly twice as great at the concentration of 50 m.e. of P_2O_5 as that to be found with the Cecil. The values for the Sharkey are also higher than those

for the Sassafras or Colts Neck. As the result of the displacement of silicate ions by the phosphate, the Sharkey colloid adsorbs considerable quantities of phosphate even though the content of free iron oxides is low. That an actual replacement occurs with all the colloids and that the silica in the supernatant liquid is not wholly the result of increased solubility of silica at low pH values may be ascertained by comparing the quantities of silica mobilized from the four colloids of different composition at approximately the same pH. For

TABLE 5

Effects of phosphation on the cation exchange capacity and ultimate pH

COLLOID	P₂O₅ ADDED AS H₃PO₄ PLUS (NH₄)₂HPO₄	P₂O₅ ADSORBED	P₂O₅ ELECTRO-DIALYZED	P₂O₅ RETAINED AFTER ELECTRO-DIALYSIS	CATION EXCHANGE CAPACITY		ULTIMATE pH
					Before electro-dialysis	After electro-dialysis	
	m.e./200 cc.	m.e./7 gm.	m.e./7 gm.	m.e./7 gm.	m.e./7 gm.	m.e./7 gm.	
Colts Neck	..	. .	....	....	....	2.33	4.90
	10 0	2 67	0.46	2 21	4 98	3.63	4 75
	20 0	3 99	0 57	2 42	5.13	3.87	3 90
	40 0	5 68	1.20	4 48	6.33	4.77	3 90
	50 0	7 22	1.20	6 02	6 30	5 11	3 85
Cecil		. .	. .	.	....	0 75	5.30
	10 0	1 05	0.19	0 96	1 31	0.95	5.10
	20 0	2.60	0 69	1 91	1 94	1 40	5 00
	40 0	3 50	0 69	2 81	2 32	1 73	4.85
	50 0	4 17	0 58	3 59	2 62	2 05	4 45
Sharkey	...	...	...	. .	. .	4.55	4.05
	10.0	2 64	1 02	1 62	6 17	4 64	4 00
	20 0	3 70	1 08	2 62	6 73	5.15	4 00
	40 0	4 61	1.50	3 11	6 97	5 17	3.90
	50 0	5 14	1.65	3 49	7 36	5 19	3.80
Sassafras	....	....	....	...	....	1.44	5.10
	10.0	1.60	0 38	1 22	2 61	1.82	4.90
	20.0	2.50	0.42	2 08	2 94	2.65	4.50
	40.0	3.46	0.64	2 82	3 61	2 81	4 00
	50.0	4 41	0.89	3 52	3 65	2 98	3.90

example at pH 4.52 with the Sharkey, 11.56 m.e. of SiO_2 was mobilized, whereas only 7.83 m.e. was mobilized at pH 4.04 from the Cecil and only 8.19 m.e. from the Sassafras at pH 4.70. The supernatant liquid of the Colts Neck, with the lowest content of silica at pH 4.98, contained 10.62 m.e. of displaced SiO_2.

Apparently the retention of phosphates by soil colloids is the result of a series of reactions. A portion of the adsorbed phosphates displaces silica,

another fraction reacts with the free iron oxide, and the remainder displaces hydroxyl groups from the uncombined basoid.

The data on the influence of phosphation upon the cation exchange capacity and alterations produced by electrodialysis upon this property are presented in table 5. The ultimate pH of the electrodialyzed phosphated clays is also tabulated at this point.

Considering first the mobilization of phosphates from the clays by electro-dialysis, it is apparent from table 5 that the Sharkey colloid even at the lowest initial treatment of P_2O_5 lost more than 1 m.e. of the retained phosphorus, whereas the values for the other clays ranged from 0.2 to 0.8 m.e. per 7 gms. The maximum loss of P_2O_5 from the Sharkey corresponds to 1.65 m.e.; that with the Colts Neck, to 1.20 m.e.; that with the Sassafras, to 0.89 m.e.; and that with the Cecil, to 0.58 m.e., per 7 gms. It is to be noted that the displaced silica (table 4) from the colloids is in the same order as the loss of phosphates by electrodialysis. The electrodialyzed phosphorus, however, does not

TABLE 6

Effect of increases in cation exchange capacity due to phosphate ion retention on formation of new exchange positions

COLLOID	INCREASE IN CATION EXCHANGE CAPACITY	MAXIMUM INCREASE IN CATION EXCHANGE CAPACITY	NEW EXCHANGE POSITION
	m.e./7 gm.	*per cent*	
Colts Neck.....................	3 97	182	$12 0 \times 10^{20}$
Cecil............................ ...	1 87	249	5.7×10^{20}
Sharkey.......................	2 81	61	8.4×10^{20}
Sassafras............................	2 21	153	6.1×10^{20}

represent completely the phosphates that displaced silicates. Undoubtedly a fraction of the phosphates adsorbed by this mechanism was mobilized, but in addition complexes formed between the free iron oxides and phosphates were ionized.

The cation exchange capacities of the phosphated clays are very interesting, and it can be seen that with the Colts Neck and Sassafras colloids the point of maximum increase in this property produced by phosphation has been attained. This point has nearly been reached with the other two colloids. Increasing adsorption of phosphates by the colloid produces progressive increases in cation exchange powers. When the colloids are compared on the basis of percentage increases in exchange powers, the order is Cecil, Colts Neck, Sassafras, and Sharkey; the percentage increases with maximum phosphation are 249, 182, 153, and 61 per cent, respectively. No correlation exists between the increase in exchange capacity and the silica-sesquioxide ratio or the content of free ferric oxides in the colloids.

When comparisons of the increases in exchange capacity due to phosphate ion retention are made on the percentage basis, it is impossible to visualize clearly the number of new exchange positions produced by this treatment. If the comparisons are made on the milliequivalent basis the indications become clearer, and when the new exchange positions are expressed in terms of molecules it is possible to differentiate distinctly the behavior of the clays with reference to the formation of new stable exchange positions. This has been done with the four colloids at maximum cation exchange increase, and the values for the new exchange positions are expressed in terms of the number of barium molecules retained by 7 gm. of the colloids in excess of the original exchange positions. The calculations are given in table 6.

The new exchange position calculations indicate that the maximum increase is to be noted with the Colts Neck colloid, and the others can be arranged in the order, Sharkey, Sassafras, and Cecil. Contrary to expectations the Sharkey, with the lowest free iron oxide content and the lowest anion exchange capacity, yielded more new exchange positions than the Cecil or Sassafras colloids that unquestionably possess more free iron oxides.

The cation exchange capacities of the phosphated electrodialyzed clays are always lower than those of the original phosphated colloids. The reduction in exchange capacity of soils has been discussed by Prince and Toth (11). These authors believe that the reduction is due to the mobilization of acidic groups during electrodialysis and the precipitation on and in the cathode chamber of ionogens that possess exchange powers. The Sharkey and Colts Neck colloids from which considerable silica had been displaced lost more new exchange positions by electrodialysis than did either the Cecil or the Sassafras. All the colloids show progressive decreases in the ultimate pH value with increasing cation exchange capacity.

MECHANISM OF PHOSPHATE RETENTION BY THE SOIL COLLOIDS

A fairly definite mechanism for phosphate adsorption by the four colloids may be postulated from the behavior of the clays during phosphation: the increases in exchange capacity, the mobilization of phosphates during electrodialysis, reduction in exchange capacity because of electrodialysis, and decreases in the ultimate pH. These facts can be linked together in elucidating the mechanisms of phosphate adsorption.

As the colloids are too complex to be represented by simple formulas, the schematic representations of Mattson (7) were chosen for depicting the behavior of the colloids. The various letters (A = acidoid, B = basoid) and the size of the letters indicate the strength and activity of the acidic and basic residues.

Colts Neck Colloid

$$\begin{matrix} \text{H} \\ \text{H} \end{matrix}\!\!>\!\!A - B\!\!<\!\!\begin{matrix} \text{OH} \\ \text{OH} \\ \text{OH} \end{matrix}$$

$$B\!-\!\begin{matrix}\text{OH}\\\text{OH}\\\text{OH}\end{matrix} + X\begin{bmatrix}\text{HPO}_4^-\\\text{H}_2\text{PO}_4^-\end{bmatrix} \rightarrow$$

$$B\!-\!\begin{matrix}\text{OH}\\\text{OH}\\\text{OH}\end{matrix}$$

$$\begin{matrix}\text{H}\\\text{H}\\\text{H}\end{matrix}\!\!>\!\!A - B = \text{PO}_4 - \text{H}$$

$$B - \text{PO}_4\!\!<\!\!\begin{matrix}\text{H}\\\text{H}\end{matrix} + X\,\text{H}_2\text{O}$$
$$\text{PO}_4 - \text{H}$$

$$B\!\!<\!\!\begin{matrix}\text{PO}_4 - \text{H}\\\text{H}\\\text{PO}_4\\\text{H}\end{matrix}$$

I   II
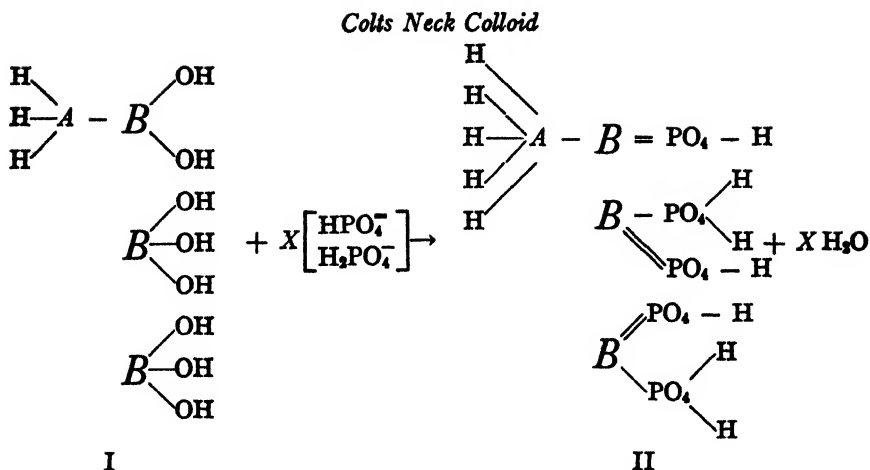
A displacement of silicate ions from the acidic residue A also occurs.

Cecil Colloid

$$\text{H} - A = B - \text{OH}$$

$$B\!-\!\begin{matrix}\text{OH}\\\text{OH}\\\text{OH}\end{matrix} + X\begin{bmatrix}\text{H}_2\text{PO}_4^-\\\text{HPO}_4^-\end{bmatrix} \rightarrow$$

$$\text{H} - A = B - \text{OH}$$
$$\text{H} \qquad\qquad + X\,\text{H}_2\text{O}$$

$$B\!\!<\!\!\begin{matrix}\text{PO}_4 - \text{H}\\\text{H}\\\text{PO}_4\\\text{H}\end{matrix}$$
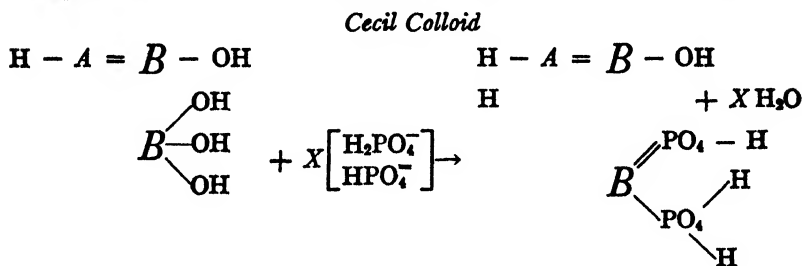
With this colloid of lower free iron oxide content, the anion adsorption capacity is less than with the Colts Neck, and the original exchange capacity is approximately one third of that exhibited by the Colts Neck. The phosphated Cecil colloid shows fewer new exchange positions than the phosphated Colts Neck colloid.

Sharkey

$$\begin{matrix}\text{H}\\\text{H}\\\text{H}\end{matrix}\!\!>\!\!A = B - \text{OH}$$

$$\begin{matrix}\text{H}\\\text{H}\end{matrix}\!\!>\!\!A = B - \text{OH}$$

$$+ X\begin{bmatrix}\text{H}_2\text{PO}_4^-\\\text{HPO}_4^-\end{bmatrix} \rightarrow$$

$$\begin{matrix}\text{H}\\\text{H}\\\text{H}\\\text{H}\end{matrix}\!\!>\!\!A = B\!\!\diagdown$$
$$\qquad\qquad\qquad\text{PO}_4-\text{H} + X\,\text{H}_2\text{O}$$
$$\begin{matrix}\text{H}\\\text{H}\\\text{H}\\\text{H}\end{matrix}\!\!>\!\!A = B\!\!\diagup$$

I   II
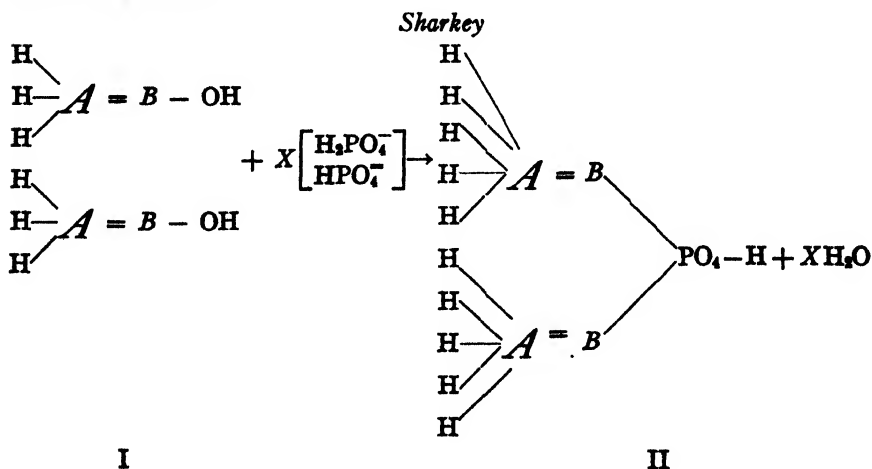
The increases in the exchange capacity with this colloid are primarily due to a displacement of silicates from the acidic residue A. Upon electrodialysis of the phosphated clay (II) a fraction of these adsorbed phosphates is mobilized with large reduction in exchange capacity. The original exchange capacity of the Sharkey is six times as great as that of the Cecil and twice that of the Colts Neck.

Sassafras

$$H - A = B - OH$$
$$H - A = B - OH$$
$$\begin{array}{c} OH \\ B-OH \\ OH \end{array} + X\begin{bmatrix} HPO_4^- \\ H_2PO_4^- \end{bmatrix} \rightarrow$$

$$H - A = B \diagdown PO_4 - H$$
$$H - A = B \diagup$$
$$\begin{array}{c} PO_4 - H \\ B \diagup \\ OH \end{array} + X H_2O$$

$$I \qquad\qquad II$$
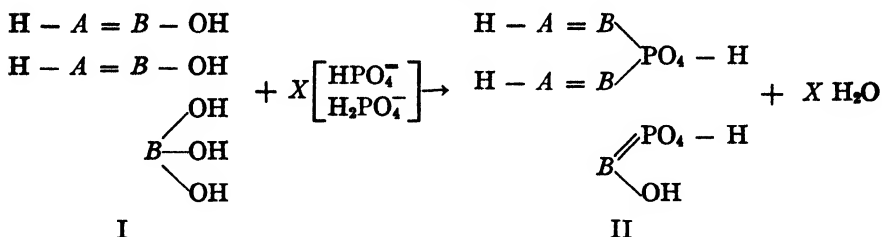
TABLE 7

Relation between the adsorption of phosphate by two soil colloids and reductions in the free iron oxide content

COLLOID	P_2O_5 ADDED AS H_3PO_4 PLUS $(NH_4)_2HPO_4$	P_2O_5 RETAINED AFTER ELECTRODIALYSIS	P_2O_5 MOBILIZED BY Na_2CO_3 TREATMENT	FREE IRON OXIDE CONTENT	CATION EXCHANGE CAPACITY	
					Phosphated colloid	Phosphated colloid with oxides removed
	m.e./200 cc.	*m.e./7 gm.*	*m.e./7 gm.*	*per cent*	*m.e./7 gm.*	*m.e./7 gm.*
Colts Neck	. .	0 00	0 75	24 80	2.33	1 42
	10 0	2 21	1 46	24 80	3.63	1.92
	20 0	3 42	1.46	24 25	3.87	2.03
	40.0	4 48	1 50	23 22	4.77	2 10
	50.0	6 02	1.55	22 59	5.11	2 30
Cecil	. . . .	0 00	trace	12 32	0 75	0.75
	10.0	0.96	0.63	12.32	0.95	0.76
	20 0	1.91	1 05	11 96	1 40	0 76
	40.0	2 82	1 25	11 13	1.73	0.79
	50.0	3 59	1.25	10 58	2.05	0 87

The original Sassafras colloid has an exchange capacity twice that of the Cecil and one third that exhibited by the Sharkey.

Lower reductions in exchange capacity with the Sassafras and Cecil colloids are due to smaller displacements of silicates by phosphates and a greater adsorption by the uncombined basoid fraction and free hydrated iron oxides.

EFFECTS OF PHOSPHATES ON THE FREE IRON OXIDE CONTENTS OF THE CECIL AND COLTS NECK COLLOIDS

The reductions in free iron oxides when the Cecil and Colts Neck colloids are treated with phosphates are presented in table 7. The formation of new

stable exchange positions is also confirmed by the data in this table.　Considerable quantities of the retained phosphates were mobilized from the phosphated electrodialyzed colloids.　This is the result of replacement of the adsorbed phosphate groups by the hydroxyl ion.　The anion displacement (phosphates by hydroxyls) may increase the free iron oxide contents of the colloids, but it is impossible to evaluate the magnitude of this effect.

The Colts Neck colloid shows a maximum reduction of 2.2 per cent in the content of free iron oxides, whereas the content of the free iron oxides in the Cecil colloid decreased 1.7 per cent.　Progressive decreases in the free iron

TABLE 8

Adsorption of silicates by soil colloids at different pH values in relation to the $\frac{SiO_2}{R_2O_3}$ ratio and free iron oxide content of the colloids

COLLOID	$\frac{SiO_2}{R_2O_3}$	FREE Fe₂O₃ CONTENT	SiO₂ ADDED AS Na₂SiO₃ PLUS HCl	pH OF SUPERNATANT LIQUID AFTER ADSORPTION	SiO₂ ADSORBED
		per cent	*m.e./200 cc.*		*m.e./7 gm.*
Colts Neck	0.81	24 80	10.0	4.92	2 80
			20.0	3.92	4.92
			40 0	3 52	9 29
			50 0	3.45	9 67
Cecil	1 01	12 32	10 0	5 30	1.94
			20 0	4 17	3.60
			40.0	3 80	5.04
			50 0	3 49	5 16
Sharkey	2.72	2.72	10 0	3 80	0 87
			20 0	3 42	1 89
			40.0	3.26	3 43
			50.0	3.18	3 22
Sassafras	3 37	7.78	10.0	4 81	1.52
			20 0	4.15	2.35
			40.0	3.70	2.87
			50 0	3.50	3 83

oxides with increasing retention of phosphates are also apparent from table 7. These reductions are the result of combination of the added phosphoric acid ions with the hydrated iron oxides.　As a result of this reaction the total free iron oxide content of the colloids decreases.　Under these conditions the mineral residues should exhibit increases in exchange capacity with the decreases in free iron oxide contents of the colloids.　The phosphated Cecil colloids after the removal of the oxides show slight but distinct increases in exchange capacity.　The data for the Colts Neck colloid also confirm the formation of stable new exchange positions.

ADSORPTION OF SILICATES BY THE VARIOUS COLLOIDS AND CHANGES IN COLLOIDAL
PROPERTIES

The data for the various systems are presented in tables 8 and 9.   As only
traces of phosphates were found in the supernatant liquid of all the series, this
point is not reported in table 8.   This behavior must mean that the displace-
ment of silicates by phosphates is very small.   It is possible that this is the
result of decreased activity of the silicate ions at acid reactions.   The adsorp-

TABLE 9

*The effects of silicate adsorptions by soil colloids and changes in the cation exchange capacity
and ultimate pH*

COLLOID	SiO₂ ADDED AS Na₂SiO₃ PLUS HCl	SiO₂ ADSORBED	CATION EXCHANGE CAPACITY		ULTIMATE pH
			Before electro-dialysis	After electro-dialysis	
	m.e./200 cc.	*m.e./7 gm.*	*m.e./7 gm.*	*m.e./7 gm.*	
Colts Neck	.	0.00	. .	2.31	4 90
	10 0	2 80	2 51	2 36	4 75
	20 0	4 92	2 63	2 39	4 65
	40 0	9 29	3 44	3.12	4 50
	50 0	9 67	3 50	3 47	4 50
Cecil		0 00	.	0.75	5 30
	10 0	1.94	0 83	0 81	5.25
	20 0	3 60	0.88	0 85	5 10
	40 0	5 04	0 91	0 87	5.10
	50 0	5 16	0 94	0 88	5.05
Sharkey	.	0 00	.	4 55	4 05
	10 0	0 87	4 55	4 55	4 05
	20 0	1.89	4 55	4 55	4 05
	40.0	3 43	4 55	4 55	4 05
	50 0	3 22	4 55	4 50	4 05
Sassafras	.	0.00		1 44	5 10
	10.0	1 52	1 53	1 51	5 00
	20 0	2 35	1 53	1 51	5 00
	40 0	2 87	1 69	1 56	4.80
	50 0	3 83	1 74	1.65	4 70

tion of the silicate ion increased with decreasing pH values and with increasing
free iron oxide content of the colloids.   The magnitude of the silicate ion
adsorption with the Cecil and Colts Neck colloids exceeds that of phosphate
ions, but with the Sharkey and Sassafras the reverse condition occurs.

The adsorption of silicate ions by the colloids has produced colloids that
have stronger acidic residues and greater exchange capacities than the original
colloidal clays.   The Sharkey colloid is the exception, however, to this state-
ment.   Electrodialysis produced smaller decreases in the exchange capacities
of the silicate-treated colloids than in those of the phosphated soil colloids.

The greater mobilization of the easily diffusible phosphate ions during electro-dialysis apparently induces more marked decreases in exchange capacity. The ultimate pH values of the silicate-treated clays that have increased exchange capacities decrease with increasing adsorption of silicates, but the decreases are not so great as those to be noted with the phosphated samples.

A comparison of the silicate-treated colloids with reference to the formation of new exchange positions and the percentage increase in exchange capacity at the highest silicate treatment shows that again the greatest number of new exchange positions are formed with the Colts Neck colloid (table 10). The adsorption of silicates by the Sharkey colloid did not result in the formation of new exchange positions, and only slight increases are to be noted with the Cecil and Sassafras colloids even though the silicate adsorption with the Cecil is greater than that with phosphates. Demolon and Batisse (3) and Mattson (9) have noted similar increases in exchange capacity when colloids are treated with silicates. The Colts Neck colloid may be taken as a typical example to express the mechanism of silicate adsorption.

TABLE 10

Effect of increases in cation exchange capacity due to silicate treatment on formation of new exchange positions

COLLOID	INCREASE IN CATION EXCHANGE CAPACITY		NEW EXCHANGE POSITIONS
	m.e./7 gm.	*per cent*	
Colts Neck................................	1.19	25 2	36 3 × 10¹⁹
Cecil..	0.19	26 4	6.0 × 10¹⁹
Sharkey....................................	0 00	0 0	0
Sassafras..........	0.30	21 0	9 1 × 10¹⁹

Colts Neck

$$\begin{array}{c} \text{H} \\ \text{H---}A\text{---}B \\ \text{H} \end{array} \begin{array}{c} \text{OH} \\ \\ \text{OH} \end{array}$$

$$B\text{---OH} + X[\text{SiO}_3 =] \rightarrow$$

$$B\text{---OH}$$

I

$$\begin{array}{c} \text{H} \\ \text{H---}A\text{---}B \\ \text{H} \end{array} \begin{array}{c} \text{OH} \\ \\ \text{OH} \end{array}$$

$$B \begin{array}{c} \text{SiO}_3\text{---H} \\ \text{SiO}_3\text{---H} \end{array}$$

$$\text{SiO}_3$$

$$B \begin{array}{c} \text{SiO}_3\text{---H} \\ \text{SiO}_3\text{---H} \end{array} + X\,\text{H}_2\text{O}$$
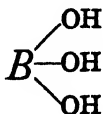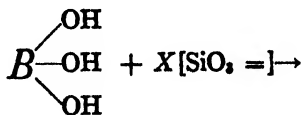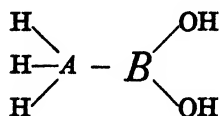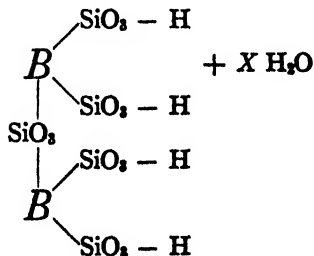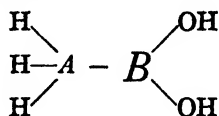
II

Formula II indicates that a reduction in free iron oxide results upon treating the colloid with silicate ions but that fewer new exchange positions are formed

by this treatment than when this colloid was phosphated. As the result of the formation of fewer new exchange positions, the ultimate pH value of the silicate-treated colloid (II) is higher than that of the phosphated colloid.

EFFECT OF SILICATES ON THE FREE IRON OXIDE CONTENTS OF THE CECIL AND COLTS NECK COLLOIDS

The fixation of silicates by Cecil and Colts Neck colloids as well as the reduction in free iron oxides are presented in table 11. The data on combined silicates indicate that with the Cecil colloid of lower free iron oxide content more of the adsorbed silicates exist as amorphous silica than in the Colts Neck colloid. As a result larger reductions in the content of the free iron oxides are to be noted with the latter colloid than with the former. Further, these data are in line with the formation of new exchange positions in the Colts Neck

TABLE 11

Adsorption of silicates by the Colts Neck and Cecil colloids and changes in free iron oxide content

COLLOID	SiO$_2$ ADDED AS Na$_2$SiO$_3$ PLUS HCl	SiO$_2$ ADSORBED	AMORPHOUS SiO$_2$	COMBINED SiO$_2$	FREE IRON OXIDE
	m.e /200 cc.	*m.e./7 gm.*	*m.e./7 gm.*	*m.e./7 gm.*	*per cent*
Colts Neck	. .	. . . .	. . . .	. . . .	24.80
	10.0	2.80	1 43	0.37	22.90
	20 0	4 92	1.93	2.99	21 32
	40.0	9 29	3 66	5 63	21.08
	50 0	9 67	3 93	5.74	20 92
Cecil	.	. . .	.	. . .	12.32
	10 0	1 94	1 49	0 45	11.69
	20 0	3.60	2.80	0.80	11.31
	40 0	5.04	3 26	1.78	10 74
	50.0	5 16	3 40	1.76	10 42

colloid. The magnitude of the free iron oxide reduction when these two colloids are treated with silicates is somewhat higher than the reductions noted with the phosphated samples. Almost 4.0 per cent reduction is attained with the Colts Neck after silicate adsorption, whereas phosphate ion adsorption by this colloid produced a decrease of only 2.2 per cent in the free iron oxides. The Cecil colloid shows the same tendency in this respect.

SUMMARY

The important rôle of the free iron oxide content of soil colloids with reference to silicate and phosphate adsorption has been elucidated. The removal of this constituent from soil colloids lessens the magnitude of phosphate adsorption and results in smaller increases in exchange capacity produced as the result of widening the acidoid–basoid ratio by the phosphate treatment.

The adsorption of phosphates by the Colts Neck, Sharkey, Cecil, and

Sassafras colloids was the result of the following series of reactions: fractions of the adsorbed phosphates displaced silicate ions from the soil colloids; another fraction reacted with the free oxides of iron; a fraction displaced hydroxyl groups from the basoid fraction.

The phosphate-treated colloids exhibited more marked increases in exchange capacity and reductions in ultimate pH than the silicate-treated colloids, even though the latter treatment generally resulted in a more marked anion adsorption by the colloids. This was explained on the basis that the activity of the silicate ions is reduced at acid pH values, and the greater adsorption of this ion, when compared to the retention of phosphates, was due to the precipitation of amorphous silica.

Adsorption of silicates and phosphates by the Colts Neck and Cecil colloids reduced the free iron oxide contents. The formation of new stable exchange groups in the two colloids was verified by the exchange capacity determinations of the colloids after the removal of the free iron oxides.

REFERENCES

(1) ANDERSON, M. S., AND MATTSON, S. 1926 Properties of the colloidal soil material. U. S. Dept. Agr. Bul. 1452.

(2) BRADFIELD, R. 1931 The Colloidal Chemistry of the Soil. Chemical Catalog Co., Inc., New York.

(3) DEMOLON, H., AND BATISSE, E. 1933 Fixation de la silici par l'argile. *Ann. Agron.* 1: 83–90.

(4) DROSDOFF, M., AND TRUOG, E. 1935 A method for removing and determining the free iron oxide in soil colloids. *Jour. Amer. Soc. Agron.* 27: 312–317.

(5) FORD, M. C. 1933 The nature of phosphate fixation in soils. *Jour. Amer. Soc. Agron.* 25: 134.

(6) MATTSON, S. 1926 Electrodialysis of the colloidal soil material and the exchangeable bases. *Jour. Agr. Res.* 33: 552–567.

(7) MATTSON, S. 1931 The laws of soil colloidal behavior: V. Ion adsorption and exchange. *Soil Sci.* 31: 311–331.

(8) MATTSON, S. 1931 The laws of soil colloidal behavior: VI. Amphoteric behavior. *Soil Sci.* 32: 343–365.

(9) MATTSON, S., AND HESTER, J. B. 1935 The laws of soil colloidal behavior: XV. The degradation and regeneration of the soil complex. *Soil Sci.* 39: 75–84.

(10) PRINCE, A. L., AND TOTH, S. J. 1936 The effects of phosphates on the cation exchange capacity of certain soils. *Soil Sci.* 42: 281–290.

(11) PRINCE, A. L., AND TOTH, S. J. 1937 Electrodialysis and cation exchange studies with soils of varying organic matter content. *Soil Sci.* 43: 205–217.

(12) TRUOG, E., AND DROSOFF, M. 1935 Determination of the mineral content of the soil adsorbing complex. *Proc. Third Internatl. Cong. Soil Sci.* 1: 92–95.

THE HIGH DEGREE OF ACCURACY OF THE IMPROVED SOIL HYDROMETER USED IN THE MECHANICAL ANALYSIS OF SOILS[1]

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In a previous paper[2] an improved type of soil hydrometer which is now used for making mechanical analysis of soils was described. This hydrometer was calibrated against soil colloidal suspensions of uniform density as well as against sulfuric acid. In the procedure for making mechanical analysis of soils by the hydrometer method, the dispersed soil is mixed with about 1 l. of distilled water, and the mixture is allowed to stand for different periods of time as a column about 13 inches high in a glass cylinder. Upon standing this column of soil suspension tends to increase in density with depth. The questions arising are: (*A*) Does the soil hydrometer, which was calibrated with suspensions or liquids of uniform density, indicate accurately the average of all the densities in the entire suspension column? (*B*) How does the amount of solid material in the entire suspension column, as indicated by the hydrometer, compare with the actual dry weight obtained when the entire suspension column is evaporated to dryness and the residue weighed? It is the purpose of this paper to present experimental data bearing upon these questions.

PROCEDURE AND EXPERIMENTAL RESULTS

Each soil was dispersed and mixed with the proper amount of distilled water in the special cylinder in the usual way[2] and allowed to stand for 1, 2, or 6 hours, thus allowing sufficient time for the suspension column to become stabilized and for the differences in densities to become more pronounced. At the end of the selected period, a hydrometer reading was taken of the undisturbed suspension column. Then by means of a rubber tube, into one end of which was inserted a curved glass tube, the entire suspension column was carefully siphoned off to within one-fourth of an inch from the solid soil column, thoroughly mixed, and its hydrometer reading taken. From this soil suspension exactly 100 cc. was taken by means of a pipette, placed in a previously weighed beaker, evaporated to dryness, and the residue weighed. Since the original entire soil suspension column was intended to contain about 1,000 cc. of water, the total amount of solids in the entire soil suspension column was easily calculated from the amount of solids found in the 100-cc. aliquot.

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[1] Journal article no. 296, new series, from the Michigan Agricultural Experiment Station.

[2] Bouyoucos, G. J. 1936 Directions for making mechanical analysis of soils by the hydrometer method. *Soil Sci.* 42: 225–229.

Table 1 shows the comparison of the total amount of soil as indicated by the hydrometer, both in the undisturbed and in the mixed suspension columns, and the total amount of soil found experimentally in these soil suspension

TABLE 1

Comparison of results obtained by the hydrometer method in undisturbed and in disturbed suspension columns, and by the gravimetric method

		AMOUNT OF CLAY IN ENTIRE SOIL SUSPENSION COLUMN		
		Indicated by hydrometer		Found by evaporating to dryness and weighing residue
SOILS	DEPTH	Column undis- turbed	Column siphoned off and mixed	
	inches	per cent	per cent	per cent
Houston clay.....	6–20	52 0	52 8	52.4
Holland sandy loam........	24–33	10.7	10 7	10.5
Sierra sandy loam.........	33–43	38 4	39 0	38.6
Carrington sandy loam.................... ..	0–6	6.5	6.0	6 0
Hagerstown silty clay loam...	6–12	75 2	75 3	75 2
Shelby silt loam............................	0–12	25.3	25.3	26.3
Nacogdoches fine sandy loam...	0–12	20.0	20 2	19 8
Davidson clay loam.........	8–20	24 4	24 4	25 1
Catalpa clay.	15–22	6 7	6 9	6.6
Oktibbeha clay..........	6–12	9 2	9 3	9.1
McKenzie clay..	0–6	10 7	10.9	10 6
Marion silt loam...	0–6	10.5	10 7	10 2
Janesville silt.....	0–3	18.5	18 6	18 8
Marshall silt loam..	0–14	21 7	21 5	21 9
Marshall silt loam.........	14–18	28 8	28.3	27.9
Miami silty clay......................	10–24	56 9	56 7	56 9
Sample of soil from Nevada	.....	29.7	29.9	30 0
Davidson loam............................	0–9	26.1	25.3	25 5
Superior fine sandy loam.................. ...	12–30	6.7	6.5	6.3
Superior fine sandy loam....................	30–40	3.0	2 7	2.5
Amarillo silty clay loam.................	10–20	41 4	39 5	39.8
Amarillo silty clay loam	70–75	40 8	40 5	40.2
Soft bauxite..............................	156–214	11.9	11.4	11.5
Houston block clay..........................	0–3	39.2	38.9	38.5
Houston block clay..........................	14–20	40.3	40.4	40.5
Houston block clay.......................	30–50	40.2	39.7	39.5
Cecil clay loam............................	5–36	30.0	29.4	28 9
Cecil clay loam.............................	36–72	8.1	8.5	8.5
Cecil clay loam............................	96–112	4.2	3.9	3.6
Sharkey clay.............................	.....	58.3	57.1	57.4
Red soil (rich in iron)........................	10–20	28.4	28.3	25.0
Muck..................................	0–12	22.2	22.4	26.4

columns by weighing the dry residue.   The results in each case are expressed as percentages of material in suspension based upon the dry weight of soil taken.   The amount of soil taken was either 10, 50, or 100 gm.

The data in table 1 show that the hydrometer takes a very accurate average

reading of all the densities in the entire soil suspension column down to the point where the solid soil column is formed. It will be seen that the amount of soil indicated by the hydrometer in the undisturbed suspension column, the density of which increases with depth, is virtually the same as that found when the entire suspension column is siphoned off and its density made uniform by mixing. The data show also that the hydrometer indicates with remarkable accuracy the amount of soil present in the entire suspension column. It is readily seen that the total amount of soil as indicated by the hydrometer is surprisingly close to the total amount that is found experimentally by evaporating the suspension column to dryness and weighing the residue. Where the disagreement is appreciable, as in the muck and the red soil rich in iron, it is due to the extreme specific gravity of these materials. But even with pure muck, which has an extremely low specific gravity, the difference in weight of material indicated by the hydrometer and that found experimentally in the evaporated residue was only about 4 per cent.

DISCUSSION

As the hydrometer takes a very accurate average reading of all the densities in the entire soil suspension column and as the amount of soil indicated by the hydrometer in the entire soil suspension column is almost exactly the same as that actually found by evaporating the entire soil suspension column to dryness and weighing the residue, it is evident that the hydrometer records conditions as they actually exist. In other words, there are no fundamental errors in the hydrometer readings such as would arise from lack of sensitivity, position of the hydrometer in the suspension column, length of the suspension column, or increase of density with the increase of depth of the suspension column. It follows that there is no fundamental reason why the hydrometer method cannot be considered accurate for making a mechanical analysis of soils. As the hydrometer itself has been shown to give reliable results, any errors in the method as a whole are those that are common and inherent in all methods of mechanical analysis, such as incomplete dispersion of the soil, use of an improper dispersing and stabilizing reagent, or of an incorrect quantity, failure to use the proper quantity of the soil sample, or incorrect assumptions from Stokes law. All the experimental evidence thus far obtained indicates that the hydrometer method is as accurate as the pipette method.

SUMMARY

The soil hydrometer when placed in a soil suspension that has been allowed to settle in order to produce differences in density with depth indicates the same quantity of soil present as that in the same suspension when it is siphoned off and mixed to produce conditions of uniform density. Furthermore, the amount indicated is the same, within limits of experimental error, as that found by gravimetric determination. These are important facts and establish conclusively that they hydrometer method is practical and accurate. At the same time they prove that certain criticisms of the method based on theoretical considerations are invalid.

SYNTHETIC SOIL AS A MEDIUM FOR THE STUDY OF CERTAIN MICROBIOLOGICAL PROCESSES[1]

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INTRODUCTION

With the exception of the tumbler and the soil plaque methods, which are based on the study of microbiological processes as a result of some specific soil treatment, most of the methods for determining microbiological processes in the soil are based largely upon the use of artificial media.

The various liquid and agar media not only differ in physical nature from the soil, but they also provide nutrients that differ in nature and concentration from those that occur in or are added to the soil. Various nutrients required by microörganisms for their activities in the soil are present partly in the mineral framework of the soil, partly in the organic and inorganic soil colloids, and partly in the soil solution; their availability depends on the physical and chemical composition of the solid part of the soil, on the composition of the soil atmosphere, on the soil solution and reaction, and on other factors.

The shortcomings of the solution and agar cultures were early recognized and were particularly emphasized by Rahn (5), who found that aeration of the soil and the thickness of the moisture film around the soil particles were two important factors regulating bacterial activity in soil. Söhngen (7), on the other hand, stressed the importance and functions of the soil colloids as affecting soil microörganisms.

Sterilized soil has been used for many studies of certain microbiological processes, but because of the changes brought about during sterilization not all the results obtained are helpful in determining the influence of various factors on microbial activity. Attempts have been made from time to time to devise a technic by which the soil microörganisms could be studied on media that would provide conditions similar to but not so complex as those that exist in the soil. Early in 1915 Allen and Bonazzi (1) made use of ignited soil in some of their work on nitrification. Conn (3), Rossi and Gesuè (6), and Cholodny (2) contributed their direct and contact-slide staining technic toward obtaining a clearer picture of how the organisms exist in the soil. Their methods, however,

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unless supplemented by physiological studies, yield little information concerning the activities of the various groups of microörganisms. Winogradsky (8) recommended the use of silica gel as a general medium for the microbiological analysis of the soil. This medium permits a parallel study of microbial growth around planted granules and of the chemical changes accompanying it. Jensen (4) made use of a synthetic soil prepared by mixing 80 per cent sand, 18.5 per cent pure kaolin, 1 per cent $CaCO_3$, and 0.5 per cent ferric oxide.

Numerous attempts have thus been made to imitate soil conditions by using various substrates, ranging from culture solutions to silica gel and more complex mixtures of sand and colloidal materials. The imperfection of most of these methods is brought out by the fact that new procedures are constantly introduced.

<div align="center">EXPERIMENTAL</div>

In the present study, an attempt has been made to prepare a synthetic medium similar to natural soil but simple enough to make possible clear interpretations of the findings. The medium decided upon was a mixture of sand and bentonite combined in proportions similar to those of sand and clay in an average loam.

A sample of fine river sand was treated with hydrochloric acid overnight and then washed with water until it was free from acid. The bentonite used was obtained from the Silica Products Company, and its particle size corresponded to that of secondary colloidal matter. Humus was prepared from soil or lowmoor peat by steaming for half an hour with 4 per cent NaOH solution, filtering, and precipitating with hydrochloric acid. The humus thus obtained was washed with water until acid free and was left overnight in contact with a solution containing 1 gm. ferric chloride, 0.1 gm. $MnSO_4$, 0.1 gm. $ZnSO_4$, and 0.1 gm. sodium molybdate. The excess of salts was then washed out with water and the resultant humus kept in suspension.

The proportion of sand to bentonite in the basic medium was 97.5:2.5. This medium was used to test its suitability as a substrate for some of the more important soil processes such as cellulose decomposition, nitrification, and nitrogen fixation.

Three series of tumblers, each containing 100-gm. portions of the following mixtures, were prepared:

Series I......Washed sand alone
        II.......97.5 per cent sand + 2.5 per cent bentonite
        III.......97.0 per cent sand + 2.5 per cent bentonite + 0.5 per cent humus

The tumblers in each series were treated as follows:

Treatment 1......Moisture only
          2......100 mgm. $(NH_4)_2HPO_4$
          3......100 mgm. $(NH_4)_2HPO_4$ + 0.5 mgm. $CaCO_3$
          4......1 gm. cellulose (ground filter paper)
          5......1 gm. cellulose (ground filter paper) and 100 mgm. $(NH_4)_2HPO_4$
          6......2 gm. glucose, 0.1 gm. K_2HPO_4, and 0.5 gm. $CaCO_3$

Optimum moisture content was maintained in all tumblers, and each received an inoculum in the form of 1 cc. of a suspension of good field soil. Incubation took place at 27°C.

TABLE 1

Cellulose decomposition and the development of fungi and bacteria in sand-bentonite media

SERIES	TREATMENT	FUNGI, THOUSANDS PER GRAM		CELLULOSE-DECOM-POSING BACTERIA, THOUSANDS PER GRAM		CELLULOSE DECOM-POSED AFTER 30 DAYS*	NITROGEN CON-SUMED*	RATIO CELLU-LOSE/N
		10 days	20 days	10 days	20 days			
						mgm.	mgm.	
I	4	40	95	<1	<1	110	....	..
I	5	66	328	10	10	460	11.5	40
II	4	1	10	10	1,000	5	.	..
II	5	12	165	10	1,000	716	16 5	43
III	4	20	70	1	10	73	....	..
III	5	355	637	1	1,000	493	15.8	31

* On the basis of 100 gm. of medium.

TABLE 2

*Influence of bentonite and humus on nitrification in sand media**

SERIES	TREATMENT	1 WEEK		2 WEEKS		4 WEEKS	
		$NO_2 - N$	$NO_3 - N$	$NO_2 - N$	$NO_3 - N$	$NO_2 - N$	$NO_3 - N$
		mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
I	2	trace	0 19	trace	0.05	trace	0.17
I	3	trace	0.15	trace	0 05	trace	0.14
II	2	trace	0.16	1.5	0 08	5 2	2.30
II	3	trace	0 24	trace	0 06	trace	0.16
III	2	trace	0 36	trace	0 08	trace	0 17
III	3	trace	0 15	trace	0 08	trace	0 15

* On the basis of 100 gm. of medium.

TABLE 3

*Influence of bentonite and humus on nitrogen fixation in sand media**

SERIES	TREATMENT	NITROGEN PER 100 GM.	AMOUNT FIXED PER 100 GM.
		mgm.	mgm.
I	1	3 2	...
I	6	11.2	8 0
II	1	4.0	...
II	6	10.0	6.0
III	1	12.8	...
III	6	14.8	2.0

* Incubation for 5 weeks.

Determinations for the numbers of fungi and cellulose-decomposing bacteria were made after an interval of 10 and 20 days. Cellulose left undecomposed

and the amount of residual nitrogen were also determined after a month's incubation (table 1). Nitrite and nitrate were determined weekly in the tumblers receiving treatments 2 and 3 (table 2). The amount of nitrogen fixed in the tumblers receiving treatment 6 was determined after an incubation of 5 weeks (table 3).

The results presented in table 1 show that the sand-bentonite medium is very favorable for the study of cellulose decomposition. The addition of humus did not improve this medium, at least as far as the particular experiment was concerned. For nitrification this medium was also found (table 2) to be better suited than sand alone; here, as well, the addition of humus was superfluous. The medium was particularly favorable for nitrite formation, but it did not favor oxidation of nitrite to nitrate. As the original reaction of the medium was about pH 9.5, the addition of $CaCO_3$ had no beneficial effect.

Only in the study of aerobic fixation of nitrogen was this medium somewhat less favorable than sand alone, as shown in table 3. This may possibly be due to the reduction in oxygen tension. Inoculations from these media into mannite solution resulted in the development of Azotobacter and in nitrogen fixation.

CONCLUSIONS

One may conclude from these results that the sand-bentonite medium has distinct advantages over artificial liquid media as well as over pure sand media for the study of certain soil microbiological processes, because it is more nearly similar to a natural soil yet it is simple in composition. Furthermore, it can be readily duplicated. The results obtained by the use of this medium can be interpreted readily in terms of soil processes.

REFERENCES

(1) ALLEN, E. R., AND BONAZZI, A. 1915 On nitrification. Preliminary observations. Ohio Agr. Exp. Sta. Tech. Bul. 7.
(2) CHOLODNY, N. 1930 Über eine neue Methode zur Untersuchung der Boden Mikroflora. Arch. Mikrobiol. 1: 620–652.
(3) CONN, H. J. 1918 The microscopic study of bacteria and fungi in soil. N. Y. Agr. Exp. Sta. Tech. Bul. 64.
(4) JENSEN, H. L. 1935 Contribution to the microbiology of Australian soils. Linn. Soc. N. S. Wales, Proc. 60, pt. 3–4.
(5) RAHN, O. 1912 Bacterial activity in soil as a function of grain size and moisture content. Mich. Agr. Exp. Sta. Tech. Bul. 16.
(6) ROSSI, G., AND GESUÈ, G. 1930 Di un nuovo indirizzo nello studio biologico del suolo. Ann. Tech. Agr. 3: 196–248.
(7) SÖHNGEN, N. L. 1913 Einfluss von Kolloiden auf microbiologische Prozesse. Centbl. Bakt. (II) 38: 621–647.
(8) WINOGRADSKY, S. 1932 Analyse microbiologique du sol, principles d'une nouvelle méthode. Ann. Inst. Pasteur 48: 89–134.

STUDIES IN SOIL HUMUS: I. ESTIMATION OF SOIL HUMUS BY OXIDATION WITH ALKALINE PERMANGANATE

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Humus in soils is a complex body, the exact nature of which depends on its origin and mode of formation. It has, however, certain specific properties which distinguish it from other natural bodies, and from the practical point of view the term "humus in soil" is comprehensible.

Estimation of humus forms an important part of any scheme of routine analysis despite the difficulties inherent in dealing with a substance of uncertain composition. From the practical point of view much can be said in favor of a simple method that gives reasonably accurate results. The uneven distribution of humus, its mode of formation, and its transitory nature lead to the conclusion that, as found in soils, it is not any one definite chemical substance but a varying mixture of several substances. Its estimation, therefore, must depend on some common property exhibited by most of its constituents.

Of the older methods loss on ignition might be mentioned as a matter of historical interest. During recent years wet combustion methods have become popular among soil scientists; of these the titration method of Walkley and Black (9) is the simplest and best known.

Apparently there is no reason why oxidation with $KMnO_4$ should not prove equally satisfactory as a means of estimating humus, especially as it was shown in a previous publication that the destruction of organic matter by $KMnO_4$ is very thorough (6). The earlier methods of estimating humus by $KMnO_4$ oxidation were based on titration of the alkaline extract. Aschman and Faber (1) extracted the soil with 5 per cent NaOH solution on a water-bath and oxidized the extract with 0.01 N $KMnO_4$. Fallot (3) modified the procedure and, as a preliminary, treated the soil with nitric acid to remove the bases. The residue was filtered, washed, and extracted with 10 per cent KOH solution for 1 hour at 100°C. Similar methods, slightly modified, were proposed by Springer (7) and by Kreulen (4). Vincent (8) showed that the nature of the alkali used for extraction of the humus influences the amount of $KMnO_4$ required for titration. More recently Nostitz (5) investigated Kotzmann's method, which consists in oxidizing 0.2 to 1 gm. of soil with 0.025 N $KMnO_4$ in 30 per cent H_2SO_4 for 15 minutes at the boiling temperature, adding excess of 0.1 N oxalic acid, and titrating residually with standard $KMnO_4$. The method was found unsuitable for soils rich in calcium carbonate.

A careful examination reveals the fact that, except for the last method, all these methods are based on the preliminary alkaline extraction of the humus. It is well known that alkalies do not extract all the humus. Besides, these experimenters have used excessive concentration of alkalies. Nostitz, on the other hand, has used an excessive amount of acid. It does not seem to have been generally recognized that excessive alkalinity or acidity can decompose $KMnO_4$, leading to erroneous results. This is illustrated in a striking manner in figure 1, in which are plotted the results of estimation of Merck's humic acid. A standard solution was estimated by $KMnO_4$ oxidation in the presence of varying amounts of NaOH or H_2SO_4. It will be seen that there is a definite range beyond which the results are erroneous with both alkaline and acid
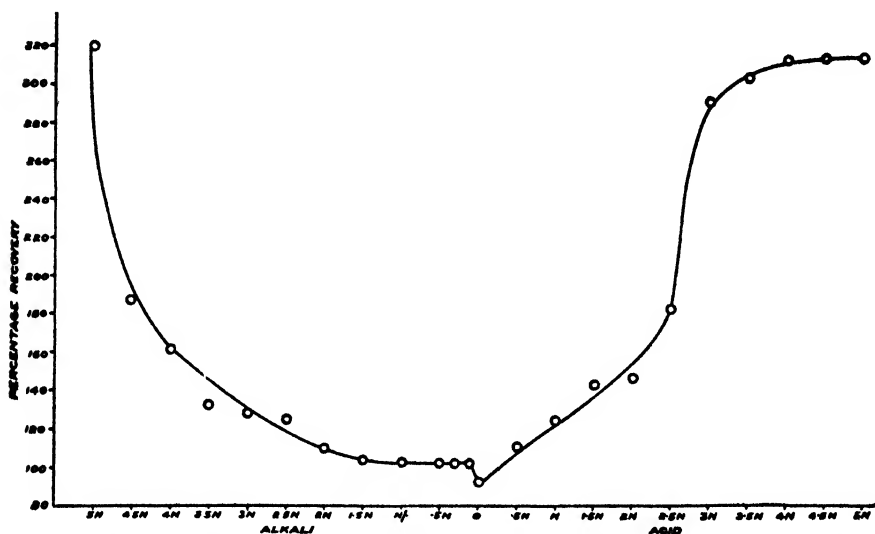
FIG. 1. RECOVERY OF HUMUS BY $KMnO_4$ OXIDATION IN ACID AND ALKALI SOLUTIONS

solutions. The failure of the $KMnO_4$ oxidation has been mainly due to failure to recognize this fact. Another important point that must be borne in mind is the concentration of $KMnO_4$. A dilute solution of $KMnO_4$, such as a 0.1 N solution, undergoes rapid decomposition in a highly alkaline medium, but a normal solution can withstand higher alkalinity without decomposing. It should be remembered that the process of oxidation with permanganate in an alkaline medium is different from that in an acid solution, as is shown in the following equations:

Acid medium $2\ KMnO_4 + 3\ H_2SO_4 \rightarrow K_2SO_4 + 2\ MnSO_4 + 3\ H_2O + 5(O)$
Alkaline medium $2\ KMnO_4 + H_2O \rightarrow 2\ MnO_2 + 2\ KOH + 3(O)$
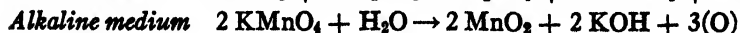
Thus larger amounts of permanganate are required for oxidation in an alkaline medium than in an acid medium.

A description of the procedure finally adopted follows:

One to two grams of the soil, depending on the amount of humus, is boiled with 20 cc. N KMnO$_4$ in 0.05 N NaOH. More of the alkaline KMnO$_4$ solu-

TABLE 1

Humus content of soils as determined by two methods

SOIL NO.	CLAY	HUMUS FOUND	
		Walkley and Black method	Alkaline KMnO$_4$ method
	per cent	per cent	per cent
6	16 00	1.86	2.12
7	13.90	2.70	3.05
8	29.80	5.00	4 50
9	9 26	3.57	3 60
10	25 26	3 57	3 65
11	19 10	2 27	2 70
12	18 34	3.84	4 12
13	8 98	2.56	2.75
14	15 80	3.24	3 27
15	25 68	2 70	3 27
16	5 84	1 68	2 20
17	14 60	3 65	3 30
18	33 28	3 70	3 65
19	27 76	6 26	6 35
20	20 74	2 12	2.65
21	8 94	8 10	7.30
22	2.32	3 25	3 32
23	16 36	3 45	3 30
24	8 86	0 61	1 13
25	15 42	2 71	2 74
26	19 58	3 25	3 33
27	11 96	2 63	2.75
28	11 90	2.07	2.34
29	10 88	2 35	2.92
30	18 78	1 28	1 54
31	8 32	1 35	1.48
32	22.90	1 69	2 07
33	15 98	2 56	2 42
34	6 90	4 19	3 65
35	22.84	2 22	2 20
36	5 38	4.59	4 19
37	8 02	4 05	4 15
38	9 56	2.08	2 38
39	12 80	1 69	1 98
40	47 04	5 81	5 25
41	48 32	3 92	3.77
42	19 10	4 38	4.19

tion is added if the color of the solution is destroyed within 10 minutes of boiling. After the oxidation is complete, the KMnO$_4$-treated material is made up to volume and filtered. To 50 cc. of the filtrate, sulfuric acid and ex-

cess of oxalic acid are added and titrated residually with $KMnO_4$. Each cubic centimeter of alkaline permanganate (N) = 3.9 mgm. of carbon.

The permanganate method as outlined is perhaps no improvement on Walkley and Black's method in regard to simplicity and rapidity, but the important point is that by two different methods of oxidation practically identical results could be obtained. Whatever the nature of humus and its various fractions, it is unlikely that we are measuring by oxidation something vastly different from the actual material. In dealing with soils one must remember that no two soils are exactly alike, and simple methods of routine analysis can furnish vast amounts of useful data in a very short time. This is the only way in which the infinite variations in soils can be evened out.

The efficacy of permanganate as an oxidizing agent according to the technic described suggests a simplification of Chakraborty's procedure for the destruction of organic matter for the mechanical analysis of soils (2). In this method the soil is first treated with alkaline permanganate. This is followed by treatment with HCl to destroy the carbonates and with HCl and sodium bisulfite to remove manganese dioxide. Leaching with sodium acetate to remove manganese sulfate follows. The soil is then dispersed with NaOH, and the clay is estimated.

This technic is not only tedious and time-consuming but makes it necessary for the operator to smell obnoxious gases during the operation. The following modification is simpler and takes less time.

Humus is destroyed by heating with alkaline permanganate. In the presence of an excess of the latter the operation is complete within 10 minutes of boiling. Excess of $KMnO_4$ is filtered, and the soil is leached with 0.2 N HCl until free from calcium. The residue is washed back in a beaker and acidified with sulfuric acid, then manganese dioxide is brought into solution by the addition of normal oxalic acid from a burette in 2-cc. lots, until the black color disappears. The soil is now leached first with 0.1 N NaCl until free from $MnSO_4$, and then with water four times, when it is ready for dispersion with NaOH.

SUMMARY

A method of estimating humus by oxidation with alkaline permanganate is outlined.

The results obtained compare favorably with Walkley and Black's method of oxidation with chromic acid.

A modification of Chakraborty's procedure for the destruction of organic matter for mechanical analysis is suggested.

REFERENCES

(1) ASCHMAN, C., AND FABER, H. 1899 Zur Bestimmung der Humussubstanz in der Ackererde. *Chem. Ztg.* 23: 61.
(2) CHAKRABORTY, J. N. 1935 The mechanical analysis of lateritic soils: III. A new method using alkaline permanganate for oxidation of organic matter. *Indian Jour. Agr. Sci.* 5: 41.

(3) FALLOT, B.   1924   Humus et humification.   Sur une methode de dosage de l'humus dans les terres.   *Chim. et Indus.* [Paris] 11: 873–874.

(4) KREULEN, D. J. W.   1931   Die Bestimmung des Humissäuregehaltes verdünnter Losüngen mittels Kaliumpermanganats.   *Brennstoff-Chem.* 12: 265–266.

(5) NOSTITZ, A. V.   1936   Determination of humus by means of potassium permanganate.   *Bodenk. u. Pflanzenernähr.* 1: 95–101.

(6) PURI, A. N., AND SARUP, ANAND   1937   The destruction of organic matter in the preliminary treatment of soils for mechanical analysis.   *Soil Sci.* 44: 87–89.

(7) SPRINGER, U.   1931   Neuere Methoden sur Untersuchung der organischen Substanz im Boden und ihre Anwendung auf Bodentypen und Humusforman.   *Ztschr. Pflanzenernähr., Düngung, u. Bodenk.* 22: 135–152.

(8) VINCENT, M.   1932   Les matières humiques des sols de Bretagne, leur dosage et leur importance comme resèrves azotées.   *Proc. Second Internatl. Cong. Soil Sci.* 3: 164–167.

(9) WALKLEY, A., AND BLACK, I. A.   1934   An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method.   *Soil Sci.* 37: 29–38.

PERCY EDGAR BROWN

Percy Edgar Brown
1885-1937

On the morning of July 8, 1937, Dr. Percy Edgar Brown, head of the Department of Agronomy at Iowa State College, died suddenly of coronary thrombosis at his home as he was preparing to leave for his office. He was 51 years old.

In the passing of Doctor Brown, Iowa State College has lost one of her ablest and most highly respected staff members; Agriculture has lost a leading educator, research worker, and administrator; and all who worked with or knew him have lost a very dear and loyal friend and helpful adviser.

Because of his thorough scientific training, his unusual ability and broad vision, his achievements brought him much honor and helped in a very large measure to bring Iowa State College to a place of honor among the leading agricultural colleges of the nation. He was a teacher of outstanding ability because of his magnetic personality, friendliness, and ability to inspire his students to master the subject under consideration. Eminently successful as a research worker in the fields of soil bacteriology, soil fertility, and soil surveying, he was recognized as an authority in this country and abroad. Few men of his age have published as many scientific articles as he. As an administrator Doctor Brown had few equals. His work was planned with a definite goal in mind; he delegated responsibility and demanded good results. He lived an exemplary life, and by virtue of his fine personality, character, and abilty he commanded the highest respect and loyalty of all who worked with him.

Doctor Brown was born on a farm at Woodbridge, New Jersey, October 9, 1885. He was graduated from Woodbridge High School in 1902 and received his Bachelor of Science degree from Rutgers College, New Brunswick, New Jersey, in 1906. From 1906 to 1910 he served as assistant soil chemist and bacteriologist at the New Jersey Agricultural Experiment Station in close association with Dr. J. G. Lipman. He received his Master of Arts degree from his Alma Mater in 1909. On many occasions Doctor Brown stated that no man had influenced his life more or given greater inspiration to his life work than had Doctor Lipman.

In 1910 Doctor Brown was appointed assistant professor of soil bacteriology at Iowa State College. Following the conferring on him of the degree of Doctor of Philosophy by Rutgers College in 1912, he was promoted to the rank of associate professor and to that of full professor in 1914. During the year 1931 Doctor Brown served as acting head of the department of agronomy. He was made head of the department in 1932.

For a number of years Doctor Brown was secretary of the agricultural

section of the American Association for the Advancement of Science and a fellow of that association.   He was a consulting editor of Soil Science, a fellow of the Iowa Academy of Science, business manager and editor-in-chief of the *Iowa State College Journal of Science*, and a member of the American Chemical Society.

At a meeting of the American Society of Agronomy at Massachusetts State College in 1920, Doctor Brown was elected to the office of secretary-treasurer, which office he held to the time of his death, except for the year 1932 when he served as president.   He was among the first ten fellows elected to the society in 1925.   The great contribution which Doctor Brown made to that organization over a long period of years has prompted J. D. Luckett, editor of the *Journal of American Society of Agronomy* to say, "I feel that the American Society of Agronomy and all that it stands for today is one of the many splendid monuments that Dr. Brown has left to his enduring memory."

Doctor Brown was president of the American Soil Survey Association in 1926; Councilor for the Society of American Bacteriologists in 1913; and expert on the National Research Council in 1918.   He was also a member of the American Organizing Committee for the first International Congress of Soil Science and one of the twelve delegates appointed to attend that convention in Washington.   After the organization of the Soil Science Society of America in 1935 he served as secretary-treasurer of both the American Society of Agronomy and the Soil Science Society of America and as treasurer of the International Society of Soil Science.

It is only natural that a man of his training and ability should have become actively affiliated with many honor societies, including Sigma Xi, Phi Kappa Phi, Alpha Zeta, Gamma Sigma Delta, Phi Lambda Upsilon, and Phi Beta Kappa.   He was also a member of the Delta Upsilon social fraternity.

For many years Doctor Brown was very active in Masonic work in the State of Iowa.   In keeping with the high standard of excellency which he maintained in every undertaking, he served diligently and faithfully every high office attainable in the several orders to which he belonged, receiving the degree of Knight Commander of the Court of Honor in 1921.   He was a Rotarian and a member of the Presbyterian church, of the Ames Chamber of Commerce, of the Ames Golf and Country Club, of the Iowa Authors Club, of the Mono Clan fraternity, and of Sons of the American Revolution.

He is survived by his mother Mrs. Jeanette E. (Walker) Brown and a sister Miss Edna Brown.

B. J. Firkins

PHYSICOCHEMICAL REACTIONS BETWEEN ORGANIC AND INORGANIC SOIL COLLOIDS AS RELATED TO AGGREGATE FORMATION[1]

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Soil organic matter has long attracted attention because of its complex and varied chemical nature and its relation to the biological and physical properties of soils. Investigations of its physicochemical aspects have been limited mainly to the study of the base exchange property, either in pure organic colloidal systems or in soils of known total organic carbon content. Knowledge of the influence of organic matter on soil granulation is based mainly on empirical observations. The need for basic information as to the influence of organic matter on soil granulation through certain cations prompted the following study in which observations were made of the changes in the colloidal properties of systems in which mineral soil colloids and organic colloids (originating from common plant residues) were allowed to react under controlled cation relationships.

Organic matter may influence the state of aggregation of a soil in one or more of several different ways. As the most active forces in rearranging soil particles probably center around the material of colloidal and smaller dimensions, it was considered that a knowledge of behavior of known combinations of organic-inorganic colloidal systems would aid in explaining the phenomenon of aggregation as it occurs in field soils and would throw some light on the rôle that organic matter plays in the formation of soil aggregates.

The investigation herein reported is an attempt to study critically the possible reactions between carefully prepared organic colloids produced in the absence of soil and the colloidal mineral fraction of soils, both in the absence and in the presence of calcium, in order to obtain a clearer understanding of the importance of calcium and organic matter in bringing about the formation of stable soil aggregates.

REVIEW OF LITERATURE

That a relationship exists between the soil organic matter and the state of aggregation of soil particles is evident from the work of several investigators (3, 5, 6, 29, 35, 36). The binding effect of organic matter in the soil and its relation to mechanical analysis has been emphasized (8, 32). A number of investigators have studied the influence of organic matter on the generation of aggregates (4, 16, 19, 24, 38). The electrolyte concentration as affected by the decomposition of organic matter was suggested by Conrad (13) as an important factor in soil granulation. The hydration effect as it relates to stable aggregation has been suggested (19, 25). The protective action of humus on clay has been widely reported (12, 14, 17, 28, 31). Further evidence of a chemical union between the organic and inorganic soil colloids has also been presented (14, 15, 27, 34, 37). The exchange capacities of organic-inorganic mixtures have been studied (1, 26, 31).

PLAN OF THE EXPERIMENTS

Preparation of material

Mineral colloids used. Colloidal fractions were prepared from three soils; namely, Putnam silt loam, Hays silty clay loam, and Davidson clay. These soils were formed under widely different environmental conditions and possess contrasting structural features. The Putnam colloid[3] had been previously isolated from the heavy B horizon of Putnam silt loam for use in other investigations. The surface soil is highly leached and is characterized by poor granulation. The Hays colloid was isolated from the surface soil of Hays silty clay loam. The sample was obtained from a cultivated field on the Fort Hays Branch Experiment Station, Hays, Kansas.[4] This soil belongs to the chernozem group and is characterized by well-developed granulation. The colloid from this soil had previously been studied by Brown and Byers (10). The lateritic Davidson colloid was isolated and characterized by Lutz (25).

The Hays colloid was isolated by a slight modification of the method commonly used in the soils laboratory of the Missouri Agricultural Experiment Station. The soil was dispersed in distilled water by means of a churn, without the aid of a peptizing agent. After standing for several days, the supernatant liquid was put through a Sharples supercentrifuge at a rapid rate. The liquid emerging was then centrifuged again with a flow rate of about 50 ml. a minute. The material collected on about the upper two-thirds of the celluloid sleeve inside the centrifuge bowl was saved and combined with that fraction obtained by concentrating the final liquid by filtering with Pasteur-Chamberland filters.

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[3] Supplied by W. A. Albrecht and T. M. McCalla.

[4] Credit is due A. L. Hallsted, associate agronomist, Division of Dry Land Agriculture, Bureau of Plant Industry, U. S. Department of Agriculture, Hays, Kansas, for supplying this sample.

The colloids so isolated were thoroughly mixed and then separated into two parts. One fraction was treated with hydrogen peroxide to remove the organic matter, following which it was electrodialyzed and standardized.[5] The second fraction was electrodialyzed and standardized without the oxidation treatment. The Putnam colloid was electrodialyzed and standardized in preparation for use. The Davidson colloid had been previously electrodialyzed. In addition to these three mineral colloids isolated from specific soil types, an electrodialyzed bentonite colloid was used.

Organic colloids used. Four different organic colloids were included in the investigation. Three of these were isolated from organic matter composts which had decomposed free of the soil, and the fourth was an ammonium hydroxide soil extract.

Compost samples were prepared from corn stover,[6] alfalfa hay, and straw. The alfalfa and straw samples were ground fine enough to pass the coarse sieve of a Wiley mill. Minerals and nitrogen were added to the straw sample; the alfalfa sample was left untreated. Both samples were kept at about the optimum moisture content for rapid decomposition by the addition of distilled water at irregular intervals. After each 30-day period the samples were dried and ground progressively finer in a Wiley mill and a ball mill. After 4 months the samples were dispersed in an excess of water, and microbial activity was stopped by the addition of toluol. Final dispersion was obtained by treating the sample with 2 per cent sodium hydroxide solution for 24 hours, followed by agitation in a barrel churn for 3 hours. The colloid fraction[7] was then isolated by the same procedure as was used for the mineral colloids except that the solution emerging from the final centrifuging was concentrated by evaporation under an electric fan at room temperature. The materials were electrodialyzed, parchment membranes being used.

The corn stover sample received essentially the same treatment as the straw but was permitted to decompose for only 2 months without being dried and reground.

The ammonium soil extract was decanted after long standing and was electrodialyzed.

Characterization of materials

As this study dealt largely with changes in properties of organic and inorganic colloids when the two colloids were mixed, it was necessary to know the properties of the original material. Some of the properties of the electrodialyzed colloidal systems are shown in table 1.

The exchange capacities of the mineral colloids were determined according to the method of Bradfield (9), which consists essentially of carbonation of the samples after the addition of calcium hydroxide, followed by a thorough

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[5] This colloid will henceforth be referred to as "Hays (H_2O_2)."

[6] Corn stover compost and ammonium hydroxide soil extract supplied by W. A. Albrecht.

[7] The organic colloids will hereafter be referred to as "corn," "alfalfa," "straw," and "soil extract," thus indicating the origin of the colloid.

aeration.   Conductivity measurements were made on the samples immediately after aeration and again after an interval of 48 hours.   Conductivity, as reciprocal ohms, was plotted against the quantity of base added.   The point where the straight lines intersected when drawn through the points thus plotted represented the exchange capacity.   The results were checked by means of pH measurements made with a glass electrode on the same samples.   The exchange capacities as found by the two methods agreed very closely.   The data reported are those obtained by the conductivity measurements at the 48-hour period of a colloid suspension of 0.25 per cent, by weight; this method gave a distinct end point.

TABLE 1

Properties of electrodialyzed colloidal materials

COLLOID SYSTEM	EXCHANGE CAPACITY OF 100 GM. OVEN-DRY COLLOID	pH VALUE* OF H-SYSTEMS	SPECIFIC GRAVITY
	m.e.		
Hays†	62	3.5	2.38‡
Hays (H_2O_2)	66	3.7	2.77
Putnam	62	3.8	2.68§
Straw	344	3.4	1.52
Corn	262	3.6	1.46
Alfalfa	291	3.4	1.68
Soil extract	314	3.9	1.68
Davidson	12		
Bentonite	89		

   * 0.25 per cent suspension by weight.
   † 2.104 per cent carbon in colloid.   Original soil contained 1.73 per cent carbon.   Both determinations by dry combustion.
   ‡ Specific gravity after dispersion with NaOH plus suction was 2.7.
   § Specific gravity established for Putnam colloid by earlier investigators.

The aspiration method could not be used so successfully on the organic colloid samples as on the mineral colloids because of the excessive foaming at the higher concentrations of calcium hydroxide.   Direct conductometric titrations with barium hydroxide were used for determining the exchange capacities of the organic matter.   Hall (18) found that exchange capacities determined by this method agreed very closely with those obtained by Bradfield's aspiration method and by the potentiometric method.

The specific gravity data were obtained by means of pycnometers and drying at 105°C.

Some very interesting differences between the Hays colloid and the Hays (H_2O_2) colloid can be noted from table 1.   The Hays has an exchange capacity of 62 m.e. per 100 gm. of oven-dry clay and a specific gravity of 2.38.   In

contrast to this, the Hays (H_2O_2) has an exchange capacity of 66 and a specific gravity of 2.77. The increase in exchange capacity due to hydrogen peroxide treatment was rather unexpected. Likewise, the difference in specific gravity is too great to be accounted for on the basis of organic matter removal. After the Hays colloid was treated with sodium hydroxide in amount slightly greater than the exchange capacity of the colloid and the system was subjected to suction, the specific gravity was found to be 2.70. The difference is probably due to the adsorbed air, which was not removed even after colloid was suspended in water for several months but was removed by hydrogen peroxide and sodium hydroxide plus suction treatments. This adsorbed air would probably also account at least partly for the lower exchange capacity of the Hays than of the Hays (H_2O_2), as on those surfaces protected by an air film, the ions could not be exchanged because of the very low dielectric constant of the air. Burgess, et al. (11) observed that some soils show an increase in exchange capacity as a result of leaching with sodium hydroxide. They explained the phenomenon as resulting probably from a formation of new soil zeolites. Just how common this phenomenon is, cannot be stated definitely on the basis of present information, but it appears that this phenomenon is probably associated with soils which are exposed to severe drying for long periods. From exchange studies on humid soils reported by many investigators the removal of organic matter has not been found to increase the exchange capacity of the colloid.

<div align="center">EXPERIMENTAL RESULTS</div>

<div align="center">*Viscosity studies*</div>

Viscosities of hydrogen-colloid mixtures. In order to determine the influence of the organic colloids on the viscosity of the inorganic colloid systems, the H-Putnam colloid[8] was combined with each of the three H-organic colloids obtained from composts, and the viscosities of the mixed systems were measured by means of an Ostwald viscosity pipette. The systems as finally diluted contained 2 per cent Putnam colloidal clay by weight. The organic colloids were added in varying quantities as percentages by weight of the clay in the system. All mixed systems stood for at least 24 hours before readings were made, and a uniform amount of material (5 ml.) was used for all viscosity readings. The apparatus was partly immersed in a constant temperature bath adjusted to 25°C.

The viscosities are reported as time in seconds required for a given quantity of the material to pass through the capillary tube of the viscosimeter. Triplicate readings, which agreed very closely with very few exceptions, were made by means of a stopwatch.

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[8] A hydrogen system, such as H-Putnam or H-alfalfa, is an electrodialyzed colloidal clay or colloidal organic matter. A calcium or sodium system, such as Ca- or Na-Putnam, is a system to which the cation was added as the hydroxide in an amount equivalent to the exchange capacity of the colloid.

In addition to the mixed organic-inorganic systems, the pure materials in equivalent concentrations were also studied. A summary of the results is presented in figure 1.

It is evident that the addition of the organic colloid to the Putnam colloid resulted in a viscosity of the resulting system quite different from that of either one of the components. This difference in viscosity cannot be accounted
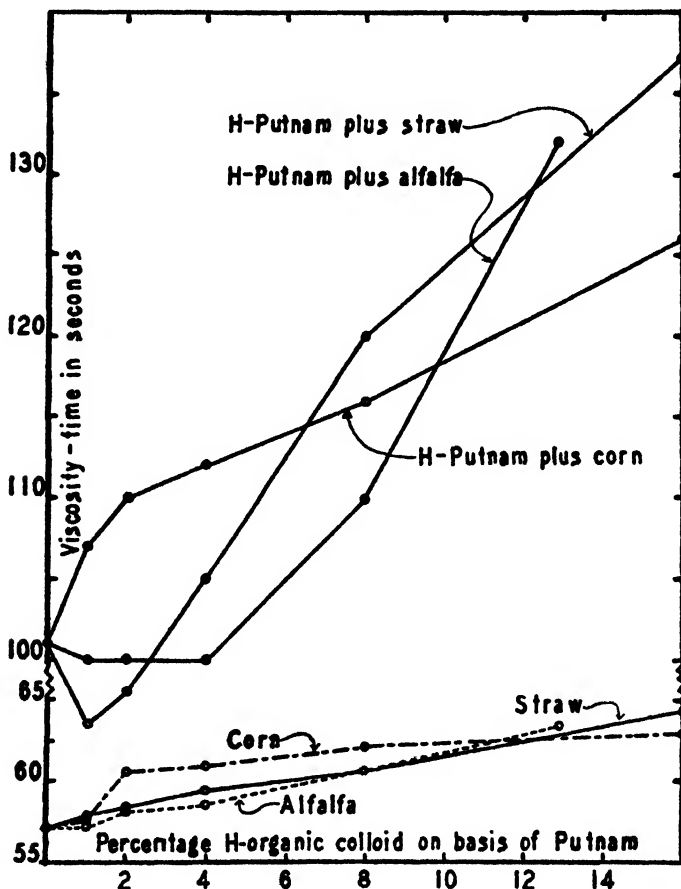
FIG. 1. VISCOSITIES OF ELECTRODIALYZED ORGANIC COLLOIDS AND THEIR EFFECTS ON THE VISCOSITY OF ELECTRODIALYZED PUTNAM COLLOIDAL CLAY

for merely by mechanical mixing, as the viscosity of the mixed systems is far from an additive result. Plotting the viscosities of the pure organic systems against their concentrations results in curves approaching straight line functions. Plotting data for organic-Putnam systems against increasing organic concentrations does not result in straight line functions. Furthermore, the two curves for any one organic system diverge with increasing quantities of organic colloid except with the lowest concentrations. The presence of small

quantities of either the H-straw or the H-alfalfa colloids lowered the viscosity of the mixed system very slightly as compared with that of the clay alone,
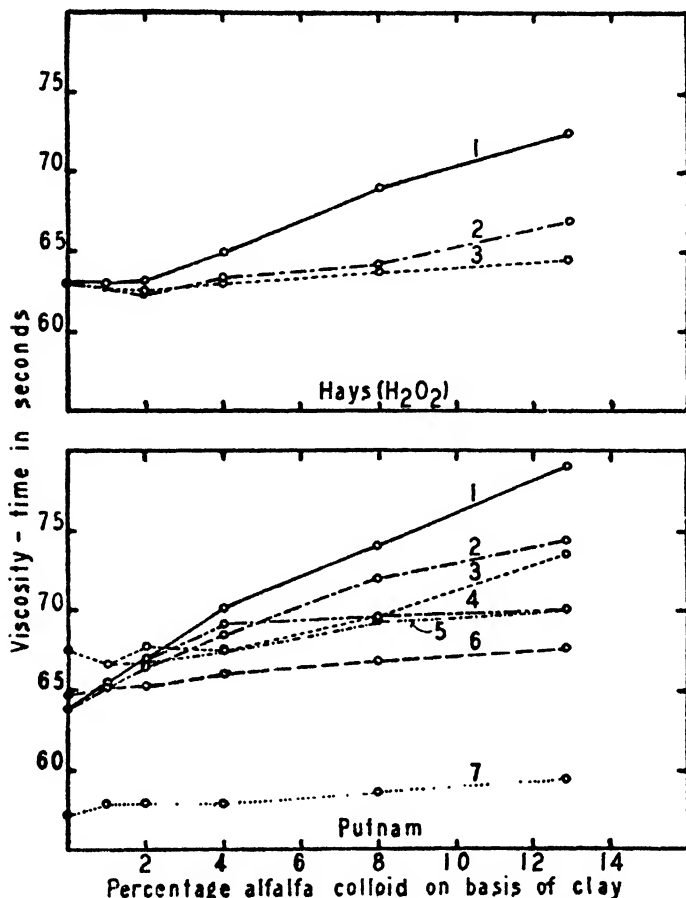
FIG. 2. VISCOSITIES OF MIXED ORGANIC-INORGANIC COLLOIDAL SYSTEMS AS INFLUENCED BY HYDROGEN, CALCIUM, AND SODIUM

The numbers indicate the type of mixing, as follows:
1. Ca-alfalfa: Ca-clay [Putnam or Hays (H_2O_2)]
2. H-alfalfa: H-clay plus Ca(OH)$_2$ equivalent to the exchange capacity of the clay.
3. H-alfalfa: H-clay.
4. H-alfalfa: Ca-Clay.
5. Ca-alfalfa: H-clay.
6. H-alfalfa: H-clay plus NaOH equivalent to the exchange capacity of the clay.
7. H-alfalfa at a concentration equal to its concentration in the mixed systems.

but higher concentrations increased markedly the viscosities of the mixtures. The H-corn colloid gave an increase in viscosity of the mixture over that of the clay alone for the entire range of organic matter additions.

As the viscosity of a system is a function of the volume occupied by the dispersed phase including water of hydration and occlusion, it would appear that the measurements reported should indicate, to some degree, the influence of the organic colloid on the dispersity of the system. If this assumption is correct then the small amounts of H-straw and H-alfalfa brought about a slight dispersion of the H-Putnam clay, but these same organic colloids at higher concentrations, and the H-corn at all concentrations studied, resulted in increased coagulation of the systems. This assumption is supported by the observations that greater amounts of H-organic colloid brought about a greater flocculation of the H-Putnam clay in the systems mentioned and in many others made up during the progress of the work.

Effect of cations on the viscosity of mixed systems. The comparative influences of calcium, sodium, and hydrogen on the viscosities of alfalfa-Putnam colloid systems at various organic concentrations were studied. The results are summarized in figure 2. The data for the two systems including sodium are almost identical; therefore, only the data for the H-alfalfa plus H-Putnam plus sodium hydroxide are shown on the graph. The same thing was also true of Ca-, Na-, and H-alfalfa systems; therefore, only the data for the H-alfalfa were plotted.

The H-alfalfa:H-Putnam system[9] in this experiment gave viscosity data of a type very similar to that of the data reported in the previous experiment (fig. 1) where the concentration of H-Putnam clay was 2 per cent. The H-Putnam clay gave a viscosity value higher than that of the Ca-Putnam clay, and the viscosity value of Na-Putnam clay was but slightly higher than that of the latter.

Where calcium was added as Ca-Putnam clay to either H-alfalfa or Ca-alfalfa and where calcium was added as calcium hydroxide to the mixed hydrogen systems, the alfalfa colloid caused slightly greater increases in viscosities than did the addition of H-alfalfa to H-Putnam. In making these comparisons, the lower initial viscosity of Ca-Putnam must be kept in mind. Where Ca-alfalfa was added to H-Putnam, the viscosities were consistently lower than those of H-alfalfa:H-Putnam systems. The greatest relative increase in viscosity occurred in the Ca-alfalfa:Ca-Putnam systems. This, as will be discussed later, is possibly due, at least partly, to the lowering of the exchange capacity of the system, resulting in the release of adsorbed calcium as calcium hydroxide. Any released calcium would increase the ion concentration in the solution and thereby reduce the zeta potential of the micellae, which in turn would cause a greater flocculation of the system.

Those systems to which sodium was added showed appreciably lower viscosity values in comparison with the other systems. It is interesting to note that the smaller amount of sodium added in the Na-alfalfa:Ca-Putnam sys-

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[9] All systems contained 1 per cent Putnam clay. The alfalfa colloid was added as percentage of the Putnam clay.

tem[10] had a slightly greater effect in reducing the viscosity values as compared to the H-alfalfa:H-Putnam plus sodium hydroxide system.

The influence of the calcium, sodium, and hydrogen ions on the viscosities of alfalfa:Hays (H_2O_2) systems[11] was also studied. The results are also summarized in figure 2. Because of the great similarity of the data for several systems, not all data are presented. The curve for the H-Hays (H_2O_2) plus H-alfalfa is essentially the same as the curves for the following systems: Ca-alfalfa plus H-Hays (H_2O_2), H-alfalfa plus Ca-Hays (H_2O_2), and Na-alfalfa plus Ca-Hays (H_2O_2).

The Ca-Hays (H_2O_2) and H-Hays (H_2O_2) possessed practically the same viscosities. The organic colloid had far less influence on the Hays (H_2O_2) than on the Putnam colloid as indicated by viscosity measurements. In spite of the lesser influence, however, the trend of the effect was essentially the same in both organic-inorganic systems. The viscosities of the alfalfa-Hays (H_2O_2) system were consistently, but very slightly, lowered by lower concentrations of the organic colloid and increased by higher concentrations. Except for the Ca-alfalfa:Ca-Hays (H_2O_2) system, the increases in viscosities were of the order which could be accounted for by mechanical mixing. As there was a consistent lowering of viscosities with lower organic additions regardless of the associated ion, however, it appears that something more than mechanical mixing has taken place.

The large increase in viscosity with increasing organic concentrations for the Ca-alfalfa:Ca-Hays (H_2O_2) system may be partly explainable on the basis of a lowered exchange capacity and consequent release of calcium ion, which would have the effect of reducing the zeta potential of the colloid.

The influence of the alfalfa colloid on the viscosities of Davidson and bentonite colloids in hydrogen and calcium systems is shown in table 2. Calcium decreased the viscosity of Davidson colloid materially as compared to the viscosity of the H-clay. H-alfalfa added to H-Davidson decreased the viscosity of the combined system even with the highest organic addition amounting to 12.89 per cent of the clay. Ca-alfalfa, on the other hand, had almost no influence on the viscosity of Ca-Davidson. The H-alfalfa not only decreased the viscosity as measured but it also noticeably increased the degree of dispersion of the system about in proportion to the quantity of organic colloid added. The H-Davidson was completely flocculated after 4 hours. After the same time interval, the H-alfalfa:H-Davidson, with the former at 8 per cent of the latter, showed in the bottom of the tube a distinct layer of accumulated floc, over which was an appreciable quantity of peptized mineral colloid. The H-alfalfa:H-Davidson with the alfalfa at 12.89 per cent of the Davidson clay was still suspended after 4 hours. Even though some floccula-

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[10] For the H-alfalfa: H-Putnam plus sodium hydroxide, 62 m.e.; the greatest addition as Na-alfalfa was 44 m.e. per 100 gm.

[11] The Hays (H_2O_2) colloid 1 per cent by weight in all systems. The alfalfa colloid was added to percentage by weight on the basis of the Hays (H_2O_2) colloid.

tion was evident, it was not sufficient to cause the formation of a layer of floc in the bottom of the tube. After 16 hours the same relative differences existed among the three systems. Although the organic-inorganic systems were more flocculated after this longer time interval than after 4 hours, still no distinct layer of floccules was formed in the system containing alfalfa colloid equivalent to 12.89 per cent of the Davidson.

The Ca-Davidson was completely flocculated after 4 hours. The Ca-alfalfa:Ca-Davidson systems appeared to be just as well flocculated as the Ca-Davidson except that a very small amount of peptized colloid remained in the supernatant liquid.

The viscosity of the Ca-bentonite was more than double the viscosity of the H-bentonite. The H-alfalfa increased the viscosity of the H-bentonite very materially, and the Ca-alfalfa decreased the viscosity of the Ca-bentonite even more markedly.

TABLE 2

Influence of cations on the viscosity of alfalfa: Davidson and alfalfa: Bentonite systems*
(Time in seconds)

CONCENTRATION OF ALFALFA COLLOID AS PERCENTAGE OF MINERAL COLLOID	0	8	12.89
H-alfalfa + H-Davidson†	71.6	66.0	65.5
Ca-alfalfa + Ca-Davidson	65.4	64.2	66.3
H-alfalfa + H-bentonite‡	146	180	180
Ca-alfalfa + Ca-bentonite	327	147	143

    * Measurements at 25°C. Distilled water 57 seconds.
    † Davidson, 1 per cent by weight.
    ‡ Bentonite, 0.55 per cent by weight.

Discussion of viscosity results. The influence of the alfalfa colloid on the inorganic colloids, as the hydrogen, calcium, or sodium systems, presents some interesting and contrasting differences as shown by viscosity measurements. In the hydrogen system the alfalfa colloid decreased the viscosity of the Davidson colloid (a lateritic type) at the higher concentration of alfalfa colloid additions. In contrast to this, the alfalfa caused a marked increase in the viscosity of the bentonite at any concentration of alfalfa additions. The Putnam was characterized by some increase in the viscosity when the higher organic colloid concentration was used but by a smaller proportionate increase than that in the bentonite. The Hays(H_2O_2) was characterized by only a very slight increase in viscosity with the higher amounts of alfalfa colloid. Both the Putnam and the Hays (H_2O_2) showed slight decreases in viscosity as a result of adding small amounts of H-alfalfa colloid.

In the calcium systems the Ca-alfalfa:Ca-bentonite showed a marked decrease in viscosity when compared to the Ca-bentonite. Ca-alfalfa:Ca-Putnam and Ca-alfalfa:Ca-Hays (H_2O_2) gave viscosity readings considerably

greater than those of the corresponding calcium inorganic colloid. On the other hand, Ca-alfalfa:Ca-Davidson gave essentially the same viscosity values as the Ca-Davidson.

From the present study it appear that these differences may be associated with types of inorganic soil colloids.

Since increased viscosity suggests increased flocculation of the colloidal micellae, the results obtained in this investigation do not confirm unqualifiedly the earlier observations that organic colloids have a marked "protective action" on the clay colloids. It is thought that the marked protective action reported by Odén (28) and by Demolon, et al. (14) is due not so much to the organic colloid but to the cation with which the organic colloid was combined. Odén added the organic matter as Na-humate, and Demolon et al. used a neutral NH_4-humate. Both cations have a marked dispersive action on colloidal clay. The same suggestion would apply to the results obtained by Buzagh (12), who studied the peptizing action of humus on kaolin in the presence of sodium hydroxide. The results of the present investigation indicate that the extent of the protective action will be determined by the amount of organic matter added, the nature of the cation combined with the organic matter, and by the type of mineral colloid used. For the Hays (H_2O_2) and Putnam colloids small amounts of organic matter caused protective action, and larger amounts had either a slight effect or a flocculating influence. For the Davidson colloid, a lateritic type, there was much more evidence of protective action by the organic colloid than for the Hays (H_2O_2) and Putnam colloids.

The results obtained indicate that the cations hydrogen, calcium, and sodium have very little differential influence on the viscosity of the alfalfa colloid.

Removal of organic matter from suspension by mineral colloids

Casual observations on the organic-inorganic systems which had stood for several days after their preparation for viscosity studies indicated a marked difference in the quantity of organic matter carried down by the flocculated material. It was simpler to determine the amount of non-flocculated organic material in the various systems than to measure the amount of organic matter in the flocculated mass. The 10-ml. samples from the viscosity studies were transferred to centrifuge tubes, allowed to stand overnight, and then centrifuged. The pure organic samples, the Putnam, the Davidson, and the bentonite samples were all centrifuged for 30 minutes at a speed of 2,000 revolutions per minute. The Hays (H_2O_2) samples were centrifuged in the same way, but because of incomplete flocculation they were centrifuged for an additional 30 minutes at 2,400 r.p.m. Even after this extra centrifuging, an appreciable amount of clay was still suspended. The supernatant liquid was then decanted. The amount of organic matter in suspension was determined by measuring the amount of 0.05 N potassium permanganate solution reduced by 5 ml. of the supernatant liquid in the presence of sulfuric acid at a tempera-

ture near the boiling point.   This method was adopted under the assumption that the mineral colloid was not oxidizable and that the organic colloid was oxidizable under such conditions.   That this assumption is probably correct is indicated by the results with supernatant liquid and from the pure mineral colloids.   The results are reported in table 3.

TABLE 3

Effectiveness of mineral colloids in the removal of organic colloids from suspension

(Data as milliliters of 0.05 N potassium permanganate reduced by organic matter per 5 ml. of supernatant liquid)*

CONCENTRATION OF ALFALFA COLLOID AS PERCENTAGE OF MINERAL COLLOID	1	2	4	8	12.89
H-alfalfa alone	0.36	0.61	1.22	2.03	3.32
Ca-alfalfa alone	0.35	0.70	1.30	2.39	4.18
Na-alfalfa alone	0.42	0.93	1.70	3.04	5.24
H-alfalfa + H-Putnam	0.04	0.08	0.12	0.18	0.20
H-alfalfa + H-Putnam + Ca(OH)₂†	0.06	0.11	0.23	0.35	0.48
Ca-alfalfa + H-Putnam	0.02	0.14	0.18	0.48	0.89
H-alfalfa + Ca-Putnam	0.04	0.12	0.22	0.56	0.90
Ca-alfalfa + Ca-Putnam	0.05	0.13	0.24	0.63	1.07
Na-alfalfa + Ca-Putnam	0.09	0.38	1.17	3.54	6.00
H-alfalfa + H-Putnam + NaOH†	1.28	1.64	2.10	3.28	5.66
H-alfalfa + H-Hays (H₂O₂)	0.40	0.64	1.13	2.19	3.25
H-alfalfa + H-Hays (H₂O₂) + Ca(OH)₂†	0.24	0.41	0.82	1.64	2.36
Ca-alfalfa + H-Hays (H₂O₂)	0.32	0.57	1.06	1.92	2.97
H-alfalfa + Ca-Hays (H₂O₂)	...	0.46	0.77	....	2.14
Ca-alfalfa + Ca-Hays (H₂O₂)	0.18	0.38	0.46	....	1.30
Na-alfalfa + Ca-Hays (H₂O₂)	0.42	0.84	2.07	3.80	8.27
H-alfalfa + H-Davidson	....	....	....	0.43	1.22
Ca-alfalfa + Ca-Davidson	....	....	....	0.54	1.26
H-alfalfa + H-bentonite	.. .	....	....	0.42	0.74
Ca-alfalfa + Ca-bentonite	....	....	....	0.95	1.51

* Materials from viscosity studies used after centrifuging.   Ca- and H-mineral colloids gave readings of 0.04 ml. KMnO₄.   Na-Putnam gave a reading of 0.28 ml.

† Base added equivalent to exchange capacity of mineral colloid.

Alfalfa:Putnam mixtures.   The results with the alfalfa:Putnam systems show that the most effective removal of the organic matter was in the H-alfalfa: H-Putnam system.   If one excludes those containing sodium, Ca-alfalfa:Ca-Putnam was the least effective in the removal of the organic matter from suspension.   The other three systems containing calcium are intermediate

between these two extremes. Of the last three systems cited, the most effective was that in which the H-alfalfa and the H-Putnam were first mixed and calcium hydroxide was added after standing. The quantity of calcium added was equal to that added in the H-alfalfa:Ca-Putnam system. Where the calcium was added as Ca-alfalfa in the Ca-alfalfa:H-Putnam system the removal of organic matter from suspension was only slightly greater than in the H-alfalfa:Ca-Putnam system, even though the actual amount of calcium added was considerably less.

The data for the systems to which sodium was added are not considered so reliable, since the larger amount of suspended inorganic colloid, which is of brown color as a sodium complex, made difficult the determination of the potassium permanganate end point.

Viscosity difference which might result in greater occlusion of organic matter by one system in comparison to other systems will not account for the differences noted. It is possible that differences in gel strength which might not be measured by a viscosimeter might account for greater occlusion of organic matter in certain systems than in others. Because similar observations have been made on mixed systems ranging in concentration of Putnam colloid from 0.25 to 2 per cent by weight, it appears that mechanical occlusion will not explain the differences. It is believed, therefore, that the differences may be due to the degree of chemical union between alfalfa and Putnam colloids. The most effective removal of organic matter from suspension occurred when both alfalfa and Putnam colloids were added as hydrogen systems, and the least effective, when both were added as calcium colloids. Also, when once the union between the hydrogen colloids was effected, the addition of calcium as calcium hydroxide appeared to disrupt the combination to a certain extent. Likewise, the presence of calcium on either phase before mixing, interfered with the union.

Alfalfa:Hays (H_2O_2) mixtures. The results with the alfalfa:Hays (H_2O_2) systems are significantly different from those obtained with corresponding Putnam systems. The Hays (H_2O_2) systems were not so well flocculated as the Putnam systems, although after centrifuging it was evident that the greater bulk of the colloidal Hays (H_2O_2) clay had been thrown down. The Ca-alfalfa:Ca-Hays (H_2O_2) was the most flocculated, but even this was not so well flocculated as the corresponding Putnam series.

When the two are mixed as hydrogen systems, it is evident that the quantity of organic matter in suspension is almost identical with that of the H-alfalfa alone. The greatest removal of organic matter occurred in the Ca-alfalfa:Ca-Hays (H_2O_2) mixture. The H-alfalfa:H-Hays (H_2O_2) to which was added calcium hydroxide in an amount equivalent to the exchange capacity of the mineral colloid gave results very similar to those for the H-alfalfa:Ca-Hays (H_2O_2) mixture. With the Ca-alfalfa:H-Hays (H_2O_2) mixture the quantity of organic matter in suspension was only slightly less than that found with these two as the hydrogen systems.

For the Hays (H_2O_2) colloid it appears that the cation is a factor in the union between the inorganic and organic colloids.

Alfalfa:Davidson and alfalfa:bentonite mixtures. With the Davidson colloid there was very little difference in the quantity of organic matter left in suspension above the flocculated hydrogen and calcium systems. For the bentonite, the hydrogen system was more effective in the removal of organic matter than was the calcium system.

These studies indicate that there was a physicochemical union between the organic and the inorganic colloids. They further indicate that calcium was not essential for the union of organic colloids with certain inorganic colloids, but that it may actually interfere with the union. On the other hand, these results indicated that no extensive union occurred between alfalfa and Hays (H_2O_2) colloids when only hydrogen systems were mixed but that calcium addition to the mixture made a combination possible. Whether other ions possess this property remains for further studies.

Exchange capacity studies

Alteration of exchange capacity of organic colloids resulting from desiccation. The removal of organic matter from the Hays colloid, according to data previously given, resulted in a slight increase in the exchange capacity. Changes in this property would suggest a compound of different nature. As calculated from the data of Anderson and Byers (2) the exchange capacity of organic matter in several soil colloids was considerably lower than the exchange capacity found for the soil extract included in this study. Since desiccation may possibly be a factor in reducing the exchange capacity of organic colloids under field conditions, this particular point was investigated.

The suspensions of organic colloids were dried at such height above a steam plate as to obtain a temperature of about 35°C. These dried samples were ground to pass a 300-mesh sieve, and two samples were removed and weighed for exchange capacity determination. In addition, two samples were taken for moisture determination and were also used for determining the exchange capacity of oven-dried material. The dried samples were moistened under reduced pressure to aid in the removal of adsorbed air. The exchange capacities were then obtained by direct conductometric titration with barium hydroxide. The dried hydrogen systems were almost completely non-dispersed. During titration with barium hydroxide the dispersion was progressively increased, but even with the largest quantity of base added the dispersion was far from complete. The exchange capacity data are summarized graphically in figure 3.

Desiccation produced only small changes in the exchange capacities of the systems. Drying at 35°C. resulted in a slight decrease (4.6 per cent) in the exchange capacity of the straw colloid, a very slight decrease for the alfalfa colloid, and a slight increase for the corn colloid. Drying at 105°C. gave decreases of 7.2 and 6.9 per cent for the straw and corn colloid, respectively, and only a very slight decrease for the alfalfa colloid. The non-dispersive

residue remaining after the dispersion of the electrodialyzed alfalfa colloid had an exchange capacity after drying at 105°C. only 10 per cent lower than that of the dispersed system. These results are in fair agreement with those reported by Powers (30), although he reported some data showing greater reductions in exchange capacities than were found in this study.

It appears that drying is an insignificant factor in altering the exchange capacity of organic colloids, provided care is exercised in obtaining thorough wetting after desiccation. Under field conditions complete wetting may never
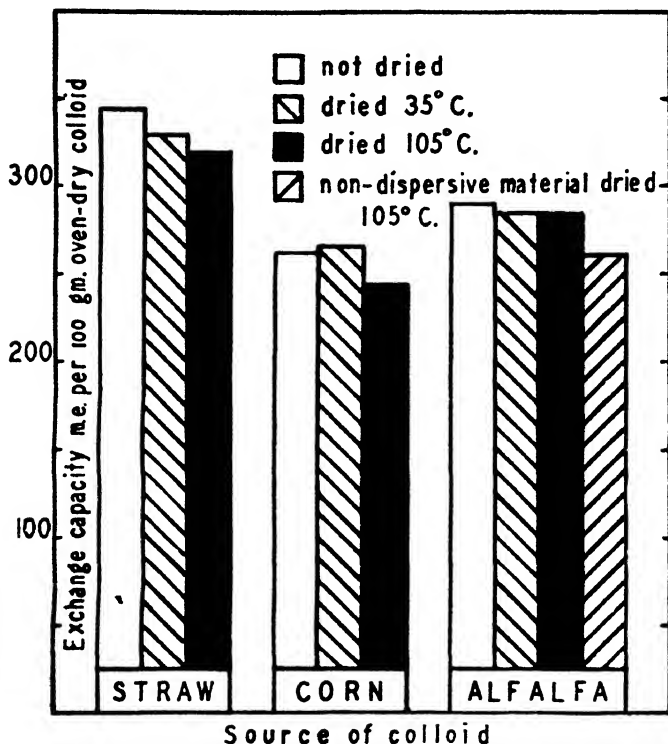
FIG 3 EXCHANGE CAPACITIES OF ORGANIC COLLOIDS AS INFLUENCED BY DESICCATION

be obtained, however, in which case the drying would be a factor in reducing the exchange capacity of organic colloidal material due to adsorbed air films.

The data also suggest that the dissiccation of pure systems of organic colloids results in the formation of a "brush heap" or open type of structure which when moistened permits the exchangeable ions to migrate in and out of the system even though the individual units are not dispersed.

Exchange capacities of organic-inorganic systems. Systems of known exchange capacities were mixed in order to determine the relation of the resulting exchange capacities of the mixed system to those of the two constituents.

Three types of organic colloids were included along with the Putnam, Hays (H_2O_2), Hays, Davidson, and bentonite colloids, and quartz, orthoclase, and electrodialyzed aluminum oxide powders. The method of determining the exchange capacities varied, depending upon the proportion of organic matter in the system. Bradfield's (9) carbonation-conductivity method was used for all systems containing not more than 56.1 per cent organic matter. For those systems containing 60, 70, 80, and 90 per cent organic matter added to clay the exchange capacities were determined potentiometrically with the glass electrode, using a series of samples with increasing proportion of calcium hydroxide. For the systems including quartz, orthoclase, and aluminum oxide, the exchange capacities were determined by direct conductometric titration with barium hydroxide.

TABLE 4

Reduction in exchange capacities resulting from mixing organic and inorganic systems

| | EXCHANGE CAPACITY PER 100 GM OF OVEN-DRIED COLLOID | | |
SYSTEM	Sum of separate exchange capacities of system	Measured exchange capacity of system	Reduction resulting from mixing
	m e	*m e*	*m e*
H-alfalfa 24.24%* + H-Putnam 75.76%	114	87	27
H-corn 24.24% + H-Putnam 75.76% .. .	110	77	33
H-straw 24.24% + H-Putnam 75.76% .	130	91	39
H-straw 24.24% + H-Hays (H_2O_2) 75.76% . .	126	86	40
H-straw 7.41 % + H-Hays 92.59%	82	68	14
H-straw 13.8% + H-bentonite 86.2% . .	124	97	27
H-straw 13.8% + H-Davidson 86.2% .	58	23	35
H-straw 39% + H-Davidson 61% .	141	76	65
H-straw 70% + feldspar powder 30% .	240	222	18
H-straw 70% + quartz powder 30%. .	240	230	10
H-straw 70% + electrodialyzed Al_2O_3 30%	240	234	6
H-straw 70% + H-Putnam 30% .	259	205	54

* All percentages by weight.

The results for a variety of systems are presented in table 4. Regardless of the type of organic and inorganic materials combined, a reduction in the exchange capacity of the system resulted from mixing. These data support those obtained by Mattson (26) and by Anderson et al. (1). The former studied aluminum and ferric "humates", and the latter studied combinations obtained by using quartz flour, Cecil soil colloid, aluminum hydroxide, and ferric hydroxide mixed with organic colloids obtained from several sources.

The exchange capacities of organic-inorganic systems with varying proportions of organic matter are shown in figure 4, together with the calculated exchange capacities. The data for the straw:Putnam and straw:Hays (H_2O_2) systems are essentially the same. Both show reductions from the calculated

values of the exchange capacities for all combinations studied. The maximum reduction with both systems occurred when the organic fraction comprised a relatively high percentage of the system. This is in contrast to the data reported by Powers (31), who found that the exchange capacity rose above that for peat colloid alone as a result of mixing certain proportions of each of two different mineral soil colloids with the organic colloid from peat. The results, however, are similar to those reported by Mattson (26) for aluminum and ferric humates. He showed that varying the proportion of humus in an Al_2O_3-humate system between 36.8 and 91.6 per cent always resulted in a reduction in the exchange capacity. The maximum reduction occurred when
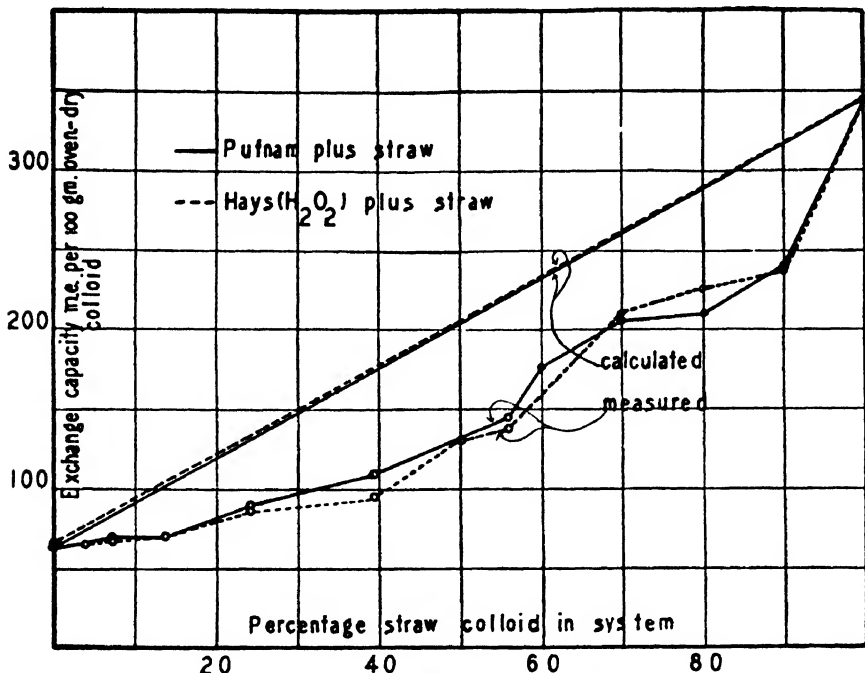
FIG. 4. EXCHANGE CAPACITIES OF SYSTEMS RESULTING FROM MIXING ORGANIC AND INORGANIC COLLOIDS

the humate comprised 68.9 per cent of the system. Mattson explains the phenomenon on the basis of the formation of an isoelectric precipitate resulting from the partial combination of the acidoid (humate) and the ampholytoid (Al_2O_3). He further assumes that the uncombined acidoid valences constitute the seat of cation adsorption.

Although the experimental results and the known characteristics of the individual compounds studied by Mattson are in agreement with his explanation, it appears that this theory is inadequate to explain the interaction of organic and inorganic soil colloids.

Discussion of exchange capacity studies. The present investigation dealt

with the combinations of different parts of acidoids in all instances except where aluminum oxide, an ampholytoid, was used. In all cases the results were qualitatively alike. In addition they are qualitatively the same as those reported by Mattson (26) and by Anderson, et al. (1). It appears, therefore, that a phenomenon characteristic of all the systems may be involved, regardless of whether an acidoid is combined with an ampholytoid or an acidoid is combined with an acidoid.

Since quartz is relatively inert chemically and is capable of an exchange reaction to only a limited extent and since it affects the exchange capacity in mixtures with organic systems in much the same way as does bentonite, which is capable of relatively great exchange reaction, some explanation other than that based on strictly chemical reactions would appear more logical. The phenomenon of polar adsorption appears to approach at least a satisfactory explanation of the reaction. The known facts conform well with the theory.

Lindau and Rhodius (23) showed that protein molecules were adsorbed by quartz in accordance with the Langmuir adsorption isotherm and were bound irreversibly. Sideri (34) expressed the belief that humus is bound with clay as a result of the selective orientation of humus particles on the clay. He reported also that the clay is able to bind humus irreversibly without previous drying. The quantity of humus accumulating on the surface greatly exceeded the amount necessary for the adsorption layer.

Surfaces possessing electrical properties polarize polar compounds. The humic compounds are polar and therefore capable of being polarized. Since they are only slightly ionized compounds, the carboxyl ends (or the end of the complex with the greatest number of carboxyl groups) would be positive. The soil colloids, bentonite, quartz, and orthoclase possess electrical properties and therefore would serve as polarizing materials. They are all electronegative and therefore would attract toward their surfaces the positive end of a polar compound. The polar adsorption should result in a close packing of the organic colloid particles on the surface of the polarizing substance. Such adsorption would result in a reduction in the exchange capacity, not because of chemical union but because of steric hindrance to the passage of the ions from the ends and sides of the organic colloids resulting from close contact either with the polarizing surface or with other polarized organic colloids. The polar adsorption not only would be a factor in reducing the exchange capacity of the organic colloid but it would also probably cover the exchangeable cations on the surface of the inorganic colloid, thus permitting only those ions within the expanding lattice to enter into the exchange reaction.

That the total surface of the inorganic material is a factor of greater importance than are its chemical nature and chemical reactivity is indicated by the similarity in the reduction in exchange capacities of the system brought about by the various inorganic colloids mixed with organic colloids when equivalent concentrations are considered. The reductions by the bentonite, Davidson, Putnam, and Hays (H_2O_2) when combined with 13.8 per cent straw

were 27, 35, 26, 30 m.e. respectively. When combined with 39 per cent straw, the reductions were 65, 62, and 79 for the Davidson, Putnam, and Hays (H_2O_2) respectively. The Davidson has an exchange capacity of 12 m.e. per 100 gm. as compared to 62 for Putnam, 66 for Hays (H_2O_2), and 89 for bentonite. The reduction for the straw:Davidson system containing 39 per cent straw was more than five times the exchange capacity of the Davidson alone. Likewise the orthoclase, quartz, and electrodialyzed aluminum oxide powders, all of which were much less finely divided than the mineral colloids, caused reductions of 18, 10, and 6 m.e. per 100 gm., respectively, in systems containing 70 per cent straw colloid. For this same concentration of straw colloid the reductions were 54 for Putnam and 51 for Hays (H_2O_2), both of which are highly dispersed colloids.

With an amphoteric compound such as aluminum hydroxide a chemical union may possibly exist between the positive valences of the compound and the negative valences of the colloidal organic acids. In addition there would probably be oriented packing around the aluminum hydroxide particles, since the organic colloidal particles, because of their relatively large size, probably would need to be packed very closely in order to satisfy the valence requirements of the positively charged particles. This close packing would also result in steric hindrance to some of the normally exchangeable ions located on the side of the organic colloids, thus lowering the exchange capacity of the system.

Not only will polar adsorption of organic matter by inorganic materials offer a fairly satisfactory explanation for reductions in exchange capacities resulting from mixing these materials, but it could also account for the other phenomena noted in this study which have resulted from the mixing of organic and inorganic materials.

Influence of organic colloids on the migration velocities of mineral soil colloids

Since changes in migration velocities in the electric field may be taken as criteria of physicochemical changes, studies were undertaken of the movement rates of the colloid mixtures. A series of organic-inorganic systems containing 0.25 per cent clay was used. The organic:Putnam systems, except those given calcium, had been made up for about 2 months as 2 per cent clay suspensions. These stock suspensions were diluted as needed. The Hays (H_2O_2) systems and the Putnam systems containing calcium had not been previously made up but were mixed, diluted, and allowed to stand for about 24 hours before cataphoretic measurements were made.

The Burton U-tube was used for making the measurements. Dilute hydrochloric acid of known specific conductivity was used above the colloidal sol and around the electrodes. A voltage of 120 with a fluctuation of not more than 4 volts was used. The final cataphoretic reading was made after 30 minutes. Most readings were made on triplicate samples. These readings

were in close agreement.   The recorded data were calculated from the mean of all readings.   The specific conductivity of all sols was measured.

The voltage drop across the sol was calculated from the following data. the specific conductivity of both the hydrochloric acid and the sol, the distance between electrodes and across the sol, and the known voltage across the entire system.   The migration velocity as microns per second per volt per centimeter was then calculated.   The results are presented in table 5.

TABLE 5

*Changes in the migration velocities of mineral colloids resulting from the addition of organic colloids**

SYSTEM	MIGRATION VELOCITY
	μ/sec./volt/cm.
H-Putnam	1.16
H-Putnam + H-straw, 16 per cent†	1.23
H-Putnam + H-straw, 8 per cent	1.8
H-Putnam + H-straw, 4 per cent..	1.9
H-Putnam + H-straw, 2 per cent .	1.71
H-Putnam + H-straw, 16 per cent + Ca(OH)₂‡ . .	1.36
Ca-Putnam	1.28
H-Putnam + H-alfalfa, 12.89 per cent .	1.34
H-Putnam + H-alfalfa, 8 per cent	2.16
H-Putnam + H-corn, 16 per cent	1.38
H-Putnam + H-corn, 8 per cent .	1.94
H-Hays (H₂O₂).	1.44
H-Hays (H₂O₂) + H-straw, 16 per cent	2.17
H-Hays (H₂O₂) + H-straw, 16 per cent + Ca(OH)₂‡ .	1.56
Ca-Hays (H₂O₂). ..	1.46
H-Hays..	1.83

* By Burton U-tube method, clay 0.25 per cent by weight.
† All percentages based on weight of clay.
‡ Ca(OH)₂ equivalent to the exchange capacity of the system.

The data obtained are rather consistent.   The organic colloid increased the rate of cataphoretic movement in every case.   The smaller amounts of organic colloids had a much greater influence on the speed of the particles than did the largest quantity used.   This was true for all three types of organic matter.   The Hays (H₂O₂) appears to be influenced more by the organic colloid than is the Putnam when hydrogen systems are considered.   The influence of concentrations of organic colloid on the migration velocity of the colloidal Putnam clay is shown graphically in figure 5.

The comparison of the migration velocities of the H-Hays, the H-Hays (H₂O₂), and the H-straw:H-Hays (H₂O₂) systems indicates that the organic matter originally present in the soil colloid has the same effect on migration velocities as has an organic colloid when added to a mineral colloid freed of organic matter by hydrogen peroxide oxidation. This suggests that the system of organic and inorganic colloid existing together naturally in soils is essentially of the same type as that made up in the laboratory by mixing a mineral colloid free from organic matter and an organic colloid obtained from compost.

The addition of calcium hydroxide to the H-straw:H-Putnam system in an amount equivalent to the exchange capacity of the system caused only a slight change in the migration velocity in comparison with that of a similar system without the calcium hydroxide. On the other hand, the saturation
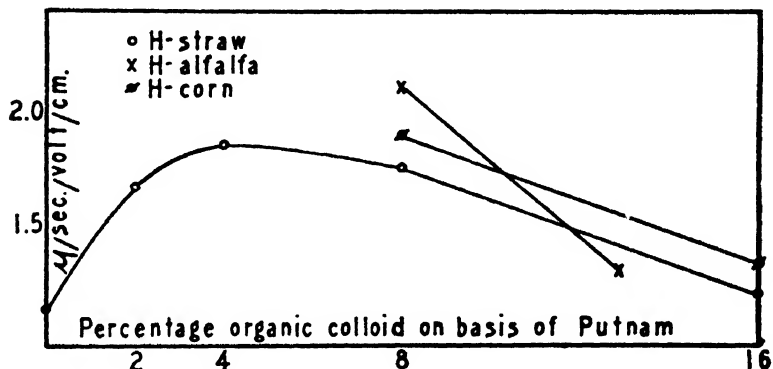
FIG. 5 CATAPHORETIC VELOCITY OF H-PUTNAM COLLOIDAL CLAY AS INFLUENCED BY THE PERCENTAGE OF HYDROGEN ORGANIC COLLOID ADDED

of the H-straw:H-Hays (H₂O₂) system with calcium resulted in a large decrease in the cataphoretic velocity as compared to that of a comparable hydrogen system.

Although the cataphoretic measurements indicated a very marked influence of organic colloids on the rate of movement of inorganic clay colloids, there was no evidence of a separation of the organic from the inorganic fractions under the influence of the electric currents. Even in those systems which moved most rapidly, the oxidation by potassium permanganate of the uppermost layer of material proved that both inorganic and organic colloids were present there and suggested that the two materials were moved as a unit.

These facts further suggest a chemical union between the organic and the inorganic fractions in the hydrogen systems as well as in the calcium systems.

Studies on aggregate formation

Influence of organic colloid and calcium ion on the aggregation of mineral colloids. The formation of aggregates as influenced by the organic colloid

and the calcium ion was studied in known systems made up of quartz or ortho-
clase powder,[12] Putnam, Hays, or Hays (H_2O_2) colloidal clay, and straw colloid.
Duplicate samples were prepared by mixing 2 gm. of powdered sand with
1 gm. of colloid (clay or straw or clay plus straw colloid) either as a calcium
system or a hydrogen system.   The combined systems were then evaporated

TABLE 6

Synthesis of aggregates as influenced by organic colloid and calcium

COLLOID SYSTEM*	KIND OF SAND	PERCENTAGE OF MATERIAL IN AGGREGATES LARGER THAN 0.1 MM.
Ca-Putnam 100%	quartz	28.5
H-Putnam 100%	quartz	33.5
H-Putnam 92% + H-straw 8%	quartz	41.0
H-Putnam 92% + H-straw 8% + Ca(OH)₂†	quartz	45.0
Ca-Putnam 92% + Ca-straw 8%	quartz	50.0
Ca-Putnam 100%	orthoclase	19.0
H-Putnam 100%	orthoclase	42.0
H-Putnam 92% + H-straw 8%	orthoclase	74.0
H-Putnam 92% + H-straw 8% + Ca(OH)₂†	orthoclase	58 0
Ca-Hays (H_2O_2) 100%	quartz	12.0
H-Hays (H_2O_2) 100%	quartz	21.0
H-Hays (H_2O_2) 92% + H-straw 8%	quartz	40.0
H-Hays (H_2O_2) 92% + H-straw 8% + Ca(OH)₂†	quartz	33.0
H-Hays (H_2O_2) 100%	orthoclase	38.0
H-Hays (H_2O_2) 92% + H-straw 8%	orthoclase	43.0
H-Hays (H_2O_2) 92% + H-straw 8% + Ca(OH)₂†	orthoclase	35.0
H-Hays‡ 100%	orthoclase	50.0
Ca-Hays 100%	orthoclase	41.0
Ca(1.5)‖ Hays 100%	orthoclase	32.0
Ca-straw 100%	quartz	71.0
H-straw 100%	quartz	94.0

* 1 gm. of colloid and 2 gm. of sand mixed.   Sand finer than 0.1 mm.

† Calcium equivalent to the measured exchange capacity of the system.

‡ Carbon content of colloid 2.104 per cent.

‖ Calcium equivalent to 1.5 times the exchange capacity.

to dryness at laboratory temperature with frequent shaking to insure thorough
mixing of the sand and colloid, following which they were alternately re-
moistened and dried three times.   Finally, the samples were oven dried,
weighed and wet sieved on a 0.1-mm. screen operated up and down in water
at a constant rate for 15 minutes.   There was no previous wetting except

[12] Ground to pass a 0.1-mm. sieve.

for the soaking of the pure straw colloid samples in water for 24 hours before sieving. That portion remaining on the screen was oven dried and weighed. This quantity represented aggregated material, since the sand had previously passed a screen of the same mesh. The final results as percentages of the system aggregated, are summarized in table 6.

During the initial drying of the samples an interesting and perhaps a fundamental difference was noted between the hydrogen and the calcium systems of the straw, the Putnam, and the straw plus Putnam mixtures. As evaporation proceeded, it was evident that the hydrogen systems held the sand in suspension much better than did the calcium systems. The hydrogen systems made a stable uniform mixture with sand, whereas the suspension was still relatively dilute. On the other hand, the calcium systems, even after they had evaporated to a very viscous state, formed a very unstable mixture with sand.

This differential effect was not evident for the Hays (H_2O_2) and for the straw plus Hays (H_2O_2) systems, since with both the hydrogen and calcium systems it was difficult to obtain a permanently uniform mixture even after the samples had evaporated nearly to dryness

The data in table 6 suggest some interesting differences. The greater effectiveness of the organic colloids either as hydrogen or calcium systems in bringing about water-stable aggregation as compared to clay colloids is clearly evident. The effectiveness of pure organic colloid is several times greater than that of an equal weight of clay colloid. In mixed organic-inorganic systems, the effect of the organic colloid is distinctly evident. These results agree well with those reported by Demolon and Henin (16) and by Baver (6). The former studied aggregate formation in systems consisting of colloidal clay, colloidal organic matter, and sand. The latter studied the degree of aggregation of a large number of soils developed under widely different environmental conditions and found that there was a statistically significant positive relationship between the organic carbon content of the soils and the degree of aggregation.

The hydrogen systems, either of clay colloids or of straw colloids alone, always resulted in a greater degree of aggregation than was true for the corresponding calcium systems. For the Hays colloid, the Ca-system was not only less effective in producing aggregation than was the corresponding H-system, but when the calcium content of the system was increased to one and one-half times the exchange capacity of the clay colloid, the degree of aggregation was further decreased. Except for the straw-Putnam colloid mixture with quartz, the mixed H-organic:H-inorganic colloid systems were aggregated to a greater extent than were the similar calcium systems.

The results indicate that calcium is no more effective in aggregate formation than is hydrogen and that it actually may be less effective than hydrogen in producing stable aggregates. These results substantiate those reported by Baver (7), who found that the hydrogen soils formed as a result of removing the calcium from many soils by acid leaching were just as well aggregated as the original soils.

The favorable influence of organic matter on the formation of water-stable aggregates is probably not due mainly to its effect on the flocculation of the clay colloid. The highly non-reversible character of the desiccated organic colloid appears to be the most important factor in determining the favorable effect of organic colloids on stable-aggregate formation. Water-stable aggregates apparently will not form without desiccation. Organic-inorganic systems prepared and allowed to stand several months flocculated almost completely, but the floccules formed were not stable, since they could be readily dispersed by agitation. That desiccation is an important factor under field conditions in promoting stable granulation through organic matter is indicated by the relatively low degree of aggregation in the A_1 horizon of the podzol, which has a relatively high organic matter content.

Stability of dried organic colloids. The idea has been expressed that the high organic matter content of rendzina soils is due to the high calcium content, which tends to stabilize the organic matter.

When the dried Ca-straw and H-straw were put into water, a very marked difference in the degree of dispersion occurred. The H-straw was almost completely non-dispersed with an almost complete absence of organic matter coloration in the supernatant liquid. The Ca-straw was dispersed to a considerable extent and caused a coffee-brown coloration of the supernatant liquid. The figures in plate 1 show differences in the degree of dispersion and in the coloration of the liquid for the two systems after the systems had stood in water for 24 hours. The H-straw (pl. 1, fig. 1) was cemented almost completely into one large compact mass in a clear supernatant liquid. The Ca-straw (pl. 1, fig. 2) was much less firmly cemented, as indicated by the more highly dispersed character of the solid fraction, and the supernatant liquid is dark colored. Even after wet sieving, the Ca-straw, on standing in water, again produced a coffee-brown coloration of the liquid. These qualitative observations confirm the results found by Hall (18).

The foregoing observations on the relative stability of Ca- and H-straw were further substantiated when the beakers in which the respective organic colloid had been dried were washed. The beakers in which Ca-straw had dried were readily cleaned by the use of water and a brush; this type of cleaning was inadequate for the breakers in which the H-straw had dried.

These results were also substantiated by the data reported by Joffe (21, 22) for the analysis of lysimeter leachates of a podzol profile. He showed that the least removal of organic matter by leaching occurred, in general, when the leachate was most acid and that the greatest loss occurred when the leachate was least acid.

The observations suggest that the high organic matter content of rendzina may not be due entirely to a direct stabilizing effect of calcium on the organic matter, since a hydrogen organic matter appears to be even more stable than the calcium system. The favorable effect of calcium on the production of plant growth, which would leave a greater organic residue in the soil, is prob-

ably an important factor in rendzina formation. The heavy character of these soils, most of which tend to support prairie vegetation instead of forest vegetation, would probably also be a contributing factor.

SUMMARY AND CONCLUSIONS

Included in this study were electrodialyzed soil colloids from Putnam silt loam, Hays silty clay loam, and Davidson clay, along with electrodialyzed organic colloids obtained from an ammonium hydroxide soil extract and from composts of straw, corn stover, and alfalfa. In addition, a bentonite colloid was used.

Studies were made of the changes in colloidal properties induced by mixing organic and inorganic colloidal systems the properties of which were known.

Organic colloidal sols altered the viscosity of colloidal clay sols to such an extent that the change could not be explained by mechanical mixing. The extent of the alteration in viscosity was influenced by the quantity of organic colloid added, the cation present, and the type of soil colloid used.

The mixing of organic colloids with soil colloids or with finely divided quartz, orthoclase, or aluminum oxide resulted in a reduction in the exchange capacity of the systems from the calculated values.

The cataphoretic velocities of colloidal clay sols were altered by the presence of organic colloids in the system. With increasing quantities of organic colloid, the migration velocity increased to a maximum value and then decreased.

There appeared to be a chemical union between the organic and the inorganic colloids. In general, the tendency to combine appeared to be most marked under acid conditions. Polar adsorption was suggested as the most probable type of chemical reaction involved.

Desiccation, when followed by thorough rewetting, had very little influence on the exchange capacity of the organic colloids studied.

Organic colloids, when saturated with either calcium or hydrogen ions, were several times more effective in cementing sand particles into water-stable aggregates than were the corresponding inorganic colloid systems.

Stable-aggregate formation apparently does not take place in the absence of dehydration.

The favorable effect of organic matter on stable aggregation was probably associated with the low degree of dispersion of the organic matter after dehydration.

Desiccated calcium organic colloid was more highly reversible in water than was desiccated hydrogen organic colloid.

REFERENCES

(1) ANDERSON, M. S., AND BYERS, H. G. 1933 Character and behavior of organic soil colloids. U. S. Dept. Agr. Tech. Bul. 377.

(2) ANDERSON, M. S., AND BYERS, H. G. 1936 Neutralization curves of the colloids of soils representative of the great soil groups. U. S. Dept. Agr Tech. Bul. 542.

(3) ANNE, PIERRE 1935 Les fumures organiques, l'état calcique et la dispersion de l'argile dans les limons. *Ann. Agron.* 5: 781-705.

(4) BARBIER, G.   1935   Influence des colloïdes humiques sur certaines propriétés physiques et chimiques des terres argileuses.   *Ann. Agron.* 5: 765–780.

(5) BAVER, L. D.   1934   The aggregation of desert soils.   *Amer. Soil. Sur. Assoc. Bul.* 16: 54.

(6) BAVER, L. D.   1934   Factors contributing to the genesis of soil micro-structure.   *Amer. Soil. Sur. Assoc. Bul.* 16: 55–56.

(7) BAVER, L. D.   1935   Aggregation of soils and calcium ion saturation.   *Amer. Soil. Sur. Assoc. Bul.* 17: 28–30.

(8) BODMAN, G. B.   1928   The H_2O_2-HCl treatment of soils as a method of dispersion in mechanical analysis.   *Soil Sci.* 26: 459–470.

(9) BRADFIELD, R., AND ALLISON, W. B.   1933   Criteria of base saturation in soils.   *Trans. Second Comn. and Alkali Subcomn. Internatl Soc. Soil Sci.* A: 63–79.

(10) BROWN, I. C., AND BYERS, H. G.   1935   The chemical and physical properties of dryland soils and their colloids.   U. S. Dept. Agr. Tech. Bul. 502.

(11) BURGESS, P. S., AND McGEORGE, W. L.   1927   Zeolite formation and base-exchange, reactions in soils.   Ariz. Agr. Exp. Sta. Tech. Bul. 156.

(12) BUZAGH, A. VON.   1927   Zur Theorie der Peptisation. III. Über die Bodenkörperregel bei der Peptisation mit hydrophilen Solen.   *Kolloid Ztschr.* 43. 220 224.

(13) CONRAD, J. P.   1934   The relation of colloid dispersion in soils to chemical changes induced by biological transformations of organic matter.   *Soil Sci* 37. 179-201.

(14) DEMOLON, A., AND BARBIER, G.   1929   Conditions de formation et constitution du complexe argilo-humique des sols.   *Compt. Rend Acad. Sci* [Paris] 188 654–656.

(15) DEMOLON, A., AND BRIGANDO, J.   1932   Sur la fixation des protéides par la sol.   *Compt. Rend. Acad. Sci.* [Paris]. 194: 311–313.

(16) DEMOLON, A., AND ILĬNIN, S.   1932   Recherches sur la structure des limone et la systhèse des agrégate.   *Soil Res.* 3   1–9.

(17) FICKENDEY, E.   1906   Notiz über Schutzwirkung von Kolloiden auf Tonsuspensionen und naturliche Tonböden.   *Jour. Landw.* 54: 343-350.

(18) HALL, N. S.   1937   Colloidal properties of soil organic matter.   Thesis, University of Missouri.

(19) HENIN, S.   1936   Étude sur la regénération des "éléments structuraux" des sols de limon.   *Ann. Agron.* 6   455–472.

(20) JENNY, HANS   1933   Soil fertility losses under Missouri conditions   Mo. Agr. Exp. Sta. Bul. 324.

(21) JOFFE, J. S.   1933   Lysimeter studies· II. The movement and translocation of soil constituents in the soil profile.   *Soil Sci.* 35: 239–257.

(22) JOFFE, J. S.   1933   Lysimeter studies III. The movement and translocation of nitrogen and organic constituents in the profile of a podzolic soil.   *Soil Sci.* 35: 401–411.

(23) LINDAU, G., AND RHODIUS, R.   1935   Die Physikalischchemische Analyse adsorbierter Eiweissmolekùle in der Grenzfläche fest-flussig.   *Ztschr. Phys. Chem* (A) 172: 321–347.

(24) LUDMILA, JAROSLAV   1936   The significance of humic materials from coal in fertilization.   *Chem. Listy* 30: 6–9. [Abs. *Chem. Abs.* 30. 3933 (1936)].

(25) LUTZ, J. F.   1934   The physico-chemical properties of soils affecting soil erosion.   Mo. Agr. Exp. Sta. Res. Bul. 212.

(26) MATTSON, SANTE   1931   The laws of soil colloidal behavior: V. Ion adsorption and exchange.   *Soil Sci.* 31: 311-331.

(27) MEYER, L.   1935   Darstellung und Verwendung von Humus-TonGemischen als Träger von Pflanzennährstoffen als Bodenverbesserungsmittel.   *Ztschr. Pflanzenernohr., Düngung, u. Bodenk.* 39: 211–244.

(28) ODÉN, SVEN   1919   Die Humisäuren.   *Kolloidchem. Beihefte* 2: 75–260.

(29) PASCHALL, A. H., BURKE, R. T. A., AND BAVER, L. D. 1934 Aggregation studies on the Muskingum, Chester, and Lansdal silt loam. *Bul Amer Soil. Sur. Assoc.* 16. 44–45.

(30) POWERS, W. L. 1932 Characteristics of dispersible organic colloids in peats. *Jour. Agr Res* 44 97–111.

(31) POWERS, W. L. 1932 Characteristics of forest soils of the northwestern United States. *Soil Sci.* 34· 1 10.

(32) ROBINSON, G. W. 1922 Note on the mechanical analysis of humus soils *Jour. Agr. Sci.* 12: 287–291.

(33) SCHLOESING, TH 1874 Détermination de l'argile dans la terre arable *Compt Rend Acad Sci.* [Paris]. 78. 1276–1279

(34) SIDERI, D. L. 1936 On the formation of structure in soil II Synthesis of aggregates; on the bonds uniting clay with sand and clay with humus. *Soil Sci* 42 461 479

(35) SOKOLOVSKY, A N. 1933 The problem of soil structure. *Trans First Comn. Internatl Soc Soil Sci* (Soviet Sect., Moscow) A, 1. 34–110

(36) TIULIN, A. 1933 Certain considerations on the genesis of soil structure and on methods for its determination. *Trans First Comn Internatl. Soc. Soil Sci* (Soviet Sect , Moscow) A, 1 111–132.

(37) WAKSMAN, S. A. 1936 Humus Origin, Chemical Composition, and Importance in Nature. The Williams & Wilkins Company, Baltimore

(38) WILLIAMS, W R. 1936 Ténacité, cohésion et structure du sol *Pedology* 5–6. 755. (Abs. in *Ann. Agron.* 6. 746.)

PLATE 1

DISPERSION OF DESICCATED ORGANIC COLLOID—QUARTZ SAND MIXTURES IN WATER

FIG. 1. H-straw colloid plus quartz sand. Note the large solid mass, which is only slightly broken down, and the absence of a dark color in the surrounding water.

FIG. 2. Ca-straw colloid plus quartz sand. Note the crumbly nature of the solid phase and the dark color of the surrounding water, resulting from the dispersion of the Ca-straw.

FIG. 1

FIG. 2

INFLUENCE OF LIGHT AND HEAT UPON THE FORMATION
OF NITRATE IN SOIL[1]

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The chemical *vs.* biological oxidation of ammonia and formation of nitrate in the soil have attracted considerable attention since this process was first recognized as one of importance in plant nutrition.   The classical investigations of Schloesing and Müntz, of Warington, of Winogradsky, and of numerous other chemists and microbiologists were generally believed to have definitely established the rôle of bacteria as the sole agents responsible for the process of nitrification in nature.   In recent years, however, new evidence, which tends to confuse the prevalent ideas, has begun to accumulate concerning the effect of light and heat upon the oxidation of ammonia

Berthelot and Gaudechon (3) demonstrated in 1910 that ammonia can be oxidized photochemically.   They were careful, however, to emphasize the fact that the oxidation takes place in solution and only to the nitrite stage. In the soil, the bacteria were still considered to be the agents responsible for the nitrification process.   Nitrates, as well, gave rise to nitrites when treated with ultra-violet light: when 100 cc. of a $0.1N$ KNO_3 solution was placed in a quartz vessel and irradiated by means of a quartz mercury lamp, 24 mgm. N_2O_3 was formed.   These results were confirmed by a number of investigators (2, 14, 22).   In all these experiments, the nitrite seemed to be the stable form into which both ammonia and nitrate were changed, as a result of photochemical action.

On the basis of these and other observations, attempts are being made to explain the natural processes of nitrification which take place in soils and in water basins; photochemical and thermochemical reactions are believed to be as important as, if not more than, the purely biological reactions in the soil, particularly in soils of tropical countries (6).   Heat and drying of soil are also said to play an important rôle in the formation of nitrate in soil (17).   According to Zolcinski (25), sunlight has an important effect upon nitrification; organic catalysts were claimed to be involved in the reaction.   Dhar reported in a series of papers, beginning in 1925 and summarized recently (6), that ammonia is gradually oxidized, in aqueous solution, to nitrite when exposed to light from a quartz mercury lamp and copper arc; the oxidation process was also found to take place in sunlight, but more slowly.   Certain salts,

notably oxides of titanium, zinc, and cadmium, acted as photosensitizers, and an alkali reaction was found to be favorable. Recent studies of ZoBell (24) and of Rakestraw and Hollaender (16) point to the rapid oxidation of ammonium salt in sea water but not in distilled water and to the nitrite stage but not the nitrate, as a result of photochemical action; the reduction of nitrate to nitrite in sea water by the action of light was also demonstrated (4).

Dhar has further found that when a large quantity of ammonium salt (5 gm.) is added to soil (500 gm.) and exposed to sunlight for 550 to 700 hours, considerable oxidation of the ammonia takes place. Although the ammonia was said to be changed to nitrite and nitrate, no substantial evidence was submitted to prove that nitrate was actually produced photochemically. Unfortunately, in an attempt to expostulate the theory that photo-nitrification is more important in soil processes than is biological oxidation, Dhar makes certain statements and generalizations that are not always substantiated by common observations. For example, he states that "the formaldehyde produced in the photo-oxidation of organic compounds present in the soil is *likely*[2] to hinder the bacterial processes"; this is interpreted as emphasizing further the minor rôle of bacteria in soil processes as compared with the action of light. The results of certain investigations carried out at the Rothamsted (18) and New Jersey Experiment Stations (21) on partial sterilization of soil are misinterpreted to indicate that "increased production of ammonia and nitrate on drying the soil, on heating it or sterilizing it by volatile antiseptics is not due to higher number of organisms in the soil," but that the resultant increase in soil surface and aeration brings about increased "oxidation of the amino acids by atmospheric oxygen leading to the liberation of ammonia and its subsequent oxidation." The following statement, which represents an attempt to interpret, in terms of photochemical processes, the more or less constant carbon-nitrogen ratio in the soil organic matter and the presumably narrower ratios found in soils of temperate climate, needs no comment: "The nitrogenous compound present in the soil of a warm country is oxidized more readily than that present in a temperate climate, just as *in fever the protein metabolism is greater than in normal health.*" The same comment may be made on the statement that "nitrates, which are *probably* the first products of nitrogen fixation, should retard the progress of nitrogen fixation in soils." This statement is not only absolutely wrong, but entirely misleading.

Corbet (5) found that ammonia may be oxidized to nitrous acid by either microbiological or chemical agencies, with the formation of hyponitrous acid as an intermediary product; nitrite was also formed from nitrate by photochemical action. The oxidation of nitrite to nitrate could take place chemically, however, only *in solution* and at *high* hydrogen-ion concentrations (pH less than 5.0), a phenomenon which is contradictory to the processes normally taking place in the soil.

[2] The italics are ours.

Fraps and Sterges (9) repeated some of the experiments of Dhar and came to the conclusion that, in normal soils, photonitrification is of little or no practical importance. Sunlight was found actually to hinder the activities of bacteria concerned in nitrate formation; the nitrite-forming organisms were more resistant, with the result that greater accumulation of nitrites than of nitrates took place in soil exposed to light. Desai and Fazal-ud-Din (7) could not obtain any photonitrification of ammonium salts in 3 months in a Punjab soil sterilized with $HgCl_2$; in unsterilized soil, 36–65 per cent of the added nitrogen was oxidized to nitrate during the same period of time; the addition of 2 per cent ZnO to soil sterilized by heat increased the photochemical oxidation of ammonia to nitrite. According to Osugi and Aoki (15), no oxidation of ammonium carbonate took place without photocatalysts, even when the carbonate was exposed to sunlight for 148 hours; ultra-violet rays, however, caused the oxidation of the ammonia, with and without photosensitizers. Zinc oxide and titanium oxide were the most active photosensitizers, and an alkaline reaction was favorable to the oxidation of the ammonium salts.

Rossi (17) did not attach much significance to the photochemical conception of nitrification in soil. He found, however, that in the uppermost layer of soil in immediate contact with the air, when nitrites and nitrates are eliminated by rainfall or irrigation, nitrates are produced at rather high temperatures (30–55°C.); this was considered to be due to a purely physicochemical reaction. No quantitative results were reported to prove that the nitrite and nitrate thus produced resulted from the oxidation of the ammonia, as there was usually a parallel increase in all three forms of nitrogen as a result of exposure of soil to the rather high temperatures.

In order to interpret the results of Rossi, it may be of interest to call attention to certain observations, made by different investigators, on the retention of nitrate in the soil and on the influence of drying upon the ease with which nitrate can be removed from the soil. Warington showed in 1882 (23) that a much greater volume of water is required to leach the nitrate from wet soil than from dry soil. King (12) demonstrated that soil particles are covered with films of water containing nitrate in solution; this nitrate is given off only slowly, by diffusion; even 10 washings with water were not sufficient to remove all the nitrate from sand, 0.8 mgm. of nitrate still remaining in 50 gm. of sand, or nearly three times that removed by the second washing. Stevens and Withers (19), as well as Allen and Bonazzi (1), obtained, by extraction with water, only a very incomplete recovery of nitrate added to the soil, especially when small amounts of nitrate were added. Gustafson (10) also found that nitrate is fixed by fine quartz sand, a smaller amount (67–80 per cent) being recovered upon addition of dilute solutions of nitrate than when concentrated (77–95 per cent) solutions were used.

Fischer (8) stated that it is a well-known fact that, with decreasing humidity, the nitrate content of the soil increases; oxidation processes were believed to take place in the dried out soils; since nitrate bacteria are sensitive to drying,

he concluded that enzymes or oxidases continue their activity even in the dry soil. Leather (13), in India, exposed a layer of soil about 0.5 inch thick to the action of the sun, whereby it became dry in 4 hours; an increased amount of nitrate was found under these conditions. Khalil (11) obtained a rapid increase in nitrate content of soil on drying, which was believed to be due to increased oxidation of ammonia.

EXPERIMENTAL

Influence of sunlight

Two soils were used in experiments on the influence of sunlight, namely, a heavy loam and an acid sandy soil. The mechanical composition and the reaction of the two soils are given in table 1. Both soils were used in an air-dry state, containing 4.5 per cent and 3.2 per cent moisture, and in a moist state, containing 12.0 per cent and 8.0 per cent moisture, respectively. Two types of containers were employed: small glazed earthenware pots, containing 1,600-gm. portions of soil, and large open glass dishes, about 14 inches in diameter, containing 2,500-gm. portions of soil.

TABLE 1

Mechanical composition and reaction of soils used

NATURE OF SOIL	SAND	SILT	CLAY	pH
	per cent	*per cent*	*per cent*	
Loam soil	36.0	34.2	29.8	6.0
Sandy soil .	55.4	26.7	17.9	4.4

Some of the pots and dishes were left uncovered and exposed to the sun; others were covered, the dishes being thoroughly blackened with enamel. Exposure to sunlight was made only on sunny days; during rainy and cloudy weather and at night, all the containers were covered. They were kept on the ground in an open space where they could have the full benefit of exposure to sun. The moisture content was adjusted daily.

The soils received, in duplicate, three different treatments, as follows: (1) No additional nitrogen; (2) $(NH_4)_2SO_4$, at the rate of 25 mgm. nitrogen, and $CaCO_3$, at the rate of 300 mgm. per 100 gm. of soil; (3) Casein, at the rate of 25 mgm. of nitrogen, and 150 mgm. $CaCO_3$, per 100 gm. of soil. As a result of these treatments, a change in reaction took place in the differently treated soils, namely, to pH 6.7 and pH 6.5 in the loam soil receiving the ammonium salt and casein respectively; the corresponding reactions for the sandy soil were pH 6.7 and pH 5.6.

The experiment was begun on July 9 and continued to October 8, 1936. The average morning and evening temperatures of the soils are given in figure 1. The first analysis was made after 3 weeks' incubation; at that time, the soils had a total exposure to sunlight of only 122 hours. The second analysis

was made after 8 weeks, with an additional exposure of 175 hours. The third analysis was made after 13 weeks, when the exposed soils had a total of 470 hours' exposure. At each sampling, the soils were thoroughly mixed, and 100-gm. portions were removed. The results for the loam soil are presented in tables 2, 3, and 4, and those for the sandy soil, in tables 5, 6, and 7.
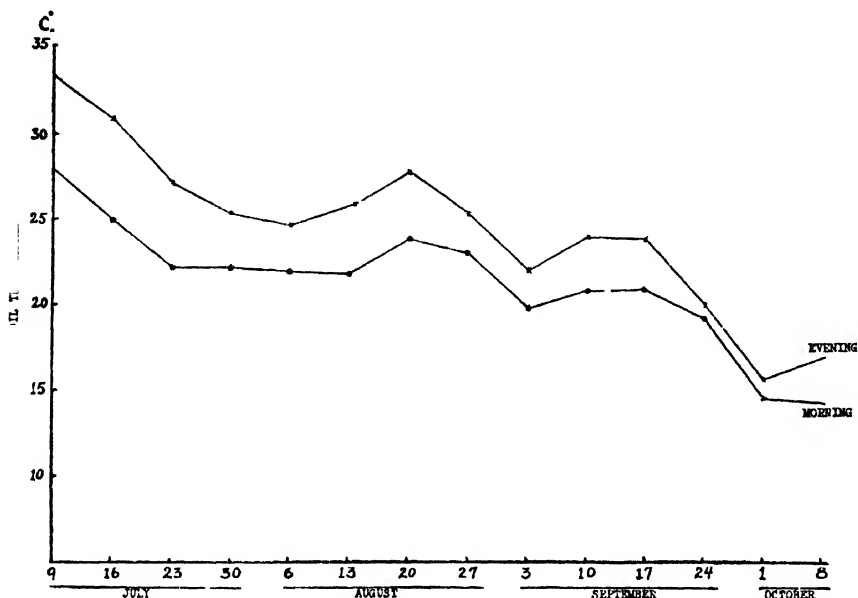
FIG. 1. SOIL TEMPERATURES THROUGHOUT SUMMER, 1936

TABLE 2

Influence of sunlight upon the process of nitrification in an air-dry loam soil kept in pots

Milligrams of nitrate in 100 gm. of soil

Incubation weeks		3		8		13	
Exposure of soil ..		Covered	Exposed	Covered	Exposed	Covered	Exposed
TREATMENT OF SOIL							
None		1.34	1.35	1 30	1.36	1.36	1.62
(NH$_4$)$_2$SO$_4$		6 48	7.75	7.05	⁷.75	17.10	30.00
Casein		2.56	3 04	2.60	3 00	2 76	3.18

No nitrification occurred in the air-dry loam soil without additional nitrogen and very little in that soil to which casein has been added; however, considerable nitrate was found in the soil receiving the ammonium salt. Air-drying of soil need not, therefore, be absolutely destructive to the process of nitrification in heavy loam soil. Although the exposed soil showed greater oxidation of the ammonia than did the soil kept in the dark, the difference did not become appreciable until after 8 weeks' incubation; the fact that

extensive formation of nitrate took place also in the covered pots indicates that the difference is not necessarily due to the effect of light only. The difference in the nitrification of the various forms of nitrogen between the moist soils exposed to the sun and the moist soils kept in the dark was very little, whether the soils were kept in pots or in glass dishes.

TABLE 3

Influence of sunlight upon the process of nitrification in a moist loam soil kept in pots

Milligrams of nitrate in 100 gm. of soil

Incubation weeks	3		8		13	
Exposure of soil	Covered	Exposed	Covered	Exposed	Covered	Exposed
TREATMENT OF SOIL						
None .	2.45	3.10	3.88	4.29	7.50	5.65
$(NH_4)_2SO_4$	17.80	19.23	22.33	20.00	35.50	33.40
Casein .	16.07	16.07	19.60	18.46	30.00	31 00

TABLE 4

Influence of sunlight upon the process of nitrification in a moist loam soil kept in glass dishes

Milligrams of nitrate in 100 gm. of soil

Incubation . . weeks	3		8		13	
Exposure of soil . . .	Covered	Exposed	Covered	Exposed	Covered	Exposed
TREATMI.NT OF SOIL						
None ..	3 38	3.65	5.31	4.91	7.23	5.74
$(NH_4)_2SO_4$	8.29	10.62	15.23	19.23	37 40	37 40
Casein	8 04	7.15	17.47	16.83	34 20	31.00

TABLE 5

Influence of sunlight upon the process of nitrification in an air-dry sandy soil kept in pots

Milligrams of nitrate in 100 gm. of soil

Incubation . . weeks	3		8		13	
Exposure of soil...	Covered	Exposed	Covered	Exposed	Covered	Exposed
TREATMENT OF SOIL						
None	2 09	2.11	2.30	2.20	2.56	2.58
$(NH_4)_2SO_4$..	2.11	2.03	2.20	2.18	2.35	2.72
Casein. .	1.94	2.40	2.16	2.43	2.29	2.20

Similar results, but with several important differences, were obtained with the sandy soils. There was virtually no nitrification in the air-dry soil. In the moist soil, the amount of nitrate produced was much less and the process was more delayed than in the loam soil. This is probably due to the acid

nature of the sandy soil. Although the acidity was corrected by the addition of $CaCO_3$, some time elapsed before conditions became favorable for the activity of the nitrifying bacteria. Variations in the amount of nitrate produced between the covered and the exposed soil did not point to any specific favorable effect of light, as shown by the fact that, in the different types of containers, the variations were not in the same direction. In the soils receiving casein, somewhat more nitrate was produced in the covered soils than in the exposed soils; these differences again were not very significant and were characteristic of experiments of this nature.

TABLE 6

Influence of sunlight upon the process of nitrification in a moist sandy soil kept in pots

Milligrams of nitrate in 100 gm. of soil

Incubation weeks	3		8		13	
Exposure of soil	Covered	Exposed	Covered	Exposed	Covered	Exposed
TREATMENT OF SOIL						
None	2.86	3.37	3 88	3 43	4 95	4.11
$(NH_4)_2SO_4$	2 24	3 04	3 87	5.61	28 00	15 70
Casein	2.18	2 52	4.07	3 64	16 00	15 40

TABLE 7

Influence of sunlight upon the process of nitrification in a moist sandy soil kept in glass dishes

Milligrams of nitrate in 100 gm of soil

Incubation weeks	3		8		13	
Exposure of soil	Covered	Exposed	Covered	Exposed	Covered	Exposed
TREATMENT OF SOIL						
None	2 76	2 51	2 60	2 85	2.67	3.62
$(NH_4)_2SO_4$	2 40	2.22	2 60	2.45	2 85	7.44
Casein .	2 07	2 30	2.15	2 58	11 66	9.60

In order to demonstrate the influence of temperature on the process of nitrification, the results of a typical experiment in which ordinary field soil was used is reported here. This soil was well-manured and limed for a number of years. One-hundred-gram portions of the soil were placed in a series of tumblers; some received $(NH_4)_2SO_4$, equivalent to 30 mgm. of nitrogen, and 0.5 gm. $CaCO_3$; others received 30 mgm. of nitrogen in the form of $NaNO_2$. The moisture was brought to optimum, namely, 60 per cent of the water-holding capacity of the soil. The tumblers were covered with glass plates and incubated, in the dark, at 8°C., 27°C., 37°C., and 55°C., in carefully regulated thermostats. At the end of different periods of incubation, the nitrite and the nitrate were determined. The results (table 8) bring out emphatically the fact that the optimum temperature for nitrification in soil is 27°C. and

that the rate of the process decreases below and above that temperature. It is particularly interesting to note that the temperature of 37°C. was at first more injurious to the nitrate-forming organisms than to the nitrite formers; as a result of this, nitrite accumulated. Later, however, the nitrate organisms seemed to have adjusted themselves to this temperature, so that, after 9 weeks, actually more nitrate was produced at 37°C. than at 27°C.; the difference, however, was small. At the highest temperature, very little if any nitrate was produced at first; however, the activities of the organisms were not depressed completely, but merely delayed. A similar delay occurred at 8°C. The optimum temperature for the oxidation of the nitrite was also 27°C.

TABLE 8

Influence of temperature upon the oxidation of ammonium sulfate and of nitrite in soil

SOIL TREATMENT	TEMPER-ATURE OF INCUBA-TION	PERIOD OF INCUBATION, IN WEEKS							
		3		5		9		11	
		NO_2-N	NO_3-N	NO_2-N	NO_3-N	NO_2-N	NO_3-N	NO_2-N	NO_3-N
	°C.	mgm	mgm	mgm	mgm	mgm	mgm	mgm	mgm
CaCO₃ only ..	27	0.02	3.0	tr.	3.46	tr.	4.44	tr.	4.44
CaCO₃ only	27	tr	2.7	tr.	3 80	tr.	5 80	tr.	5.85
(NH₄)₂SO₄ + CaCO₃.	8	0.12	2.0	0.09	3.42	0.12	16.0	tr	17.14
(NH₄)₂SO₄ + CaCO₃	27	0.27	34.28	0.02	26 66	tr.	24 0	tr.	24.00
(NH₄)₂SO₄ + CaCO₃	37	22.50	8.88	9.28	16.00	tr.	26.6	tr.	21 80
(NH₄)₂SO₄ + CaCO₃	55	0.27	1 0	1 29	2 58	0.24	12.63	tr.	15 00
NaNO₂ .	8		1.71		2 68		3.60		3.87
NaNO₂	27		4.80		6 00		17.14		17.14
NaNO₂	37		5.00		4.80		12.64		12.63
NaNO₂	55		4.50		3 46		6.60		7.06

Influence of drying of soil

A series of experiments were now conducted to test the effect of drying and leaching of soil upon the formation of nitrate. A well-manured and limed soil was used for this purpose. The reaction of this soil was pH 6.8, and the organic matter content was 2.35 per cent, calculated from the organic carbon by the use of the factor 1.724. Twenty-gram portions of the air-dry soil were leached thoroughly with 90 cc. of water and placed in thermostats, kept at 50° and 65°C., for 48 hours. They were than leached again with 70 cc. water, and the nitrate was determined in the leachings. Some of the soils then received 25-mgm. portions of (NH₄)₂SO₄. All the soil portions were placed in the thermostats and kept at the aforementioned temperatures for another 48 hours; they were then leached again, and the nitrate was determined. The leaching process was repeated at different intervals. Nitrite was also determined but was usually found in mere traces. The results presented in table 9 show that the ammonium sulfate had no effect upon the

amount of nitrate found on leaching dried soils. One must conclude from these results that no oxidation of ammonia took place in the dried soils kept at 50° and 65°C. The source of nitrate found in the soil on repeated drying and leaching must, therefore, be in the soil itself. A similar soil incubated under optimum moisture conditions at 28°C. gave, per 100 gm. of soil, in 20 days, 19.2 mgm. nitrate nitrogen, in the presence of added ammonium salt; when no source of nitrogen was added, 3.33 mgm. of nitrate nitrogen was produced from the organic nitrogen of the soil.

This experiment was repeated, using 40-gm. portions of soil. To some portions of soil 100-mgm. portions of dried blood were added; to others, 50-mgm. portions of $(NH_4)_2SO_4$; and to still others, 20 mgm. $NaNO_3$. The soils

TABLE 9

Influence of leaching and drying of soil upon its nitrate content

Milligrams of NO_3-N in 100 gm. of soil

Temperature of drying	50°C.		65°C.	
LENGTH OF DRYING	No added nitrogen	25 mgm $(NH_4)_2SO_4$	No added nitrogen	25 mgm $(NH_4)_2SO_4$
days				
2	0.21	0.22	0.22	0.23
4	0.16	0.10	0 16	0.09
8	0.17	0.15	0.20	0.26
13	0.15	0 18	0.20	0.28
20	0 16	0.17	0.22	0.20
27	0 09	0 08	0 10	0.09
42*	0.69	0.52	0.34	0 34
78*	0 22	0.25	0.21	0.20
Total	1 85	1.67	1.65	1.69

* In previous leachings, the soil was not removed from the paper on which it was dried, however, in these leachings, the soil was removed, thoroughly stirred with water, and filtered.

were kept at 50° and 65°C., were leached at different intervals, and nitrate was determined. In view of the small differences obtained at the two different temperatures, only the averages are reported. Some of the soils receiving no nitrogen and $NaNO_3$ were dried for 7 days before they were first leached. The results of this experiment show again (table 10) that no oxidation of the ammonium salt or of the organic nitrogen took place in dried soil. The nitrate added to the soil (8 mgm. nitrogen per 100 gm. of soil) and leached immediately was fully recovered, whereas the nitrate added to the soil and allowed to dry for 7 days was not fully recovered. These results also disprove any formation of nitrate by oxidation as a result of heating or drying the soil; they merely indicate better recovery of the nitrate under these conditions.

A more detailed experiment was conducted on the fixation of nitrate in

soil.   Different quantities of nitrate and ammonium sulfate $+$ CaCO$_3$ were
added to soil portions.   Some were leached immediately, and the leachings
were repeated after drying for 5-day periods at 50°C.   Others were adjusted
to optimum moisture and incubated at 27°C. for 10 and 21 days; they were
then leached, dried, and leached again.   The results presented in table 11
tend to confirm those of previous experiments.   The ammonia was not oxidized
in the dry soil.   Only the nitrate, whether added or previously formed as a
result of biological oxidation, was leached out.   After the first few leachings,
the quantity of nitrate found in the soil after each 5-day drying period ranged
from 0.08 to 0.18 mgm. per 100 gm. of soil.   When this quantity is calculated
on an acre basis, it reaches the figure of 1.6 to 3.6 pounds.   If, under natural
conditions, a similar amount were produced in the field as a result of drying,
it might be sufficient to supply the need of plants.   When one considers,

TABLE 10

Influence of drying upon the formation of nitrate from organic and inorganic forms of nitrogen
Milligrams of nitrate-N in 100 gm. of soil

PERIOD OF INCUBATION	NO ADDITIONAL NITROGEN		DRIED BLOOD ADDED	(NH₄)₂SO₄ ADDED	NaNO₃ ADDED	
	Series 1	Series 2*			Series 1	Series 2
2 hours	1.34		2.31	0.95	9.06	
24 "	0.32		0.34	0.21	0.80	
48 "	0.20		0.23	0.23	0.31	
7 days	0.11	0.85	0.10	0.21	0 25	6.38
12 "	0.25	0.08	0.28	0 30	0.27	0.83
20 "	0.08	0 08	0.08	0.11	0 08	0 08
30 "	0 12	0.11	0.12	0 12	0.10	0.13
46 "	0.13	0.13	0.12	0.11	0 12	0 13
Total	2.55	1.25	3.58	2.24	10 99	7 55

* Series 2 was not leached for 7 days.

however, the limitations of experiments of this nature, especially the very
small quantities of nitrate involved, one is hardly justified in generalizing
from results thus obtained and in applying them to field conditions.

As small amounts of nitrate are found in most of the reagents employed and
in the water used, it was essential to determine to what extent this nitrate
could account for the nitrate found in the soil on repeated drying and leaching.
Another experiment was therefore set up, in which three soils from the ferti-
lizer series of plots of the New Jersey Agricultural Experiment Station were
used; namely, plots 5A, 11A, and 18B.   Plot 5A receives yearly applications
of stable manure, 11A receives ammonium sulfate, and 18B receives manure,
sodium nitrate, and lime.   Plot 11A has now become so acid that very little
nitrification takes place in that soil, although some nitrate is usually found.
The samples of soil were taken on April 20, or 12 days after the fertilizer was

applied. There was only little rainfall during that period, which accounts for the high amount of nitrate found in soil 18B. Four 30-gm. portions of

TABLE 11

Fixation of nitrate in soil

Milligrams of NO_3-N in 100 gm. of soil

PERIOD OF DRYING	NO NITROGEN ADDED	NaNO₃ ADDED			(NH₄)₂SO₄ + CaCO₃ ADDED		
days		5 mgm. N	12 5 mgm N	25 mgm. N	5 mgm N	12 5 mgm. N	25 mgm N
				Leaching begun immediately			
0*	1.15	6.47	14 06	24.40	1.18	0.85	0.79
5*	0.24	0 26	1.25	1.69	0.29	0.26	0.28
10*	0 16	0.18	0.13	0 24	0.15	0.16	0 16
15*	0.09	0.11	0.11	0.11	0.09	0.09	0.08
20*	0.38	0 35	0.31	0.29	0.37	0.38	0.35
25	0 08	0.10	0.10	0 10	0 10	0 10	0.10
30	0 10	0.09	0 10	0 10	0.10	0.09	0.10
Total	2 20	7.56	16 06	26.93	2.28	1 93	1 86
				Leaching begun after 10 days' incubation at 27°C.			
1*	1.50	7.70	15 50	26 90	3.84	3.22	2.50
5*	0 09	0 19	0.30	0.87	0.13	0.14	0.10
10*	0.34	0.37	0.51	0 50	0.46	0.38	0 37
15	0 08	0 10	0.10	0.10	0.08	0.08	0.08
20	0.12	0.09	0.12	0 08	0 10	0.12	0.12
25	0 15	0.13	0.13	0.13	0.15	0.15	0.13
30	0 16	0.17	0 17	0.18	0 13	0 18	0.17
Total	2.44	8.75	16.83	28.76	4.89	4.27	3.47
				Leaching begun after 21 days' incubation at 27°C.			
1	6 46	12.65	16.40	26.25	11.53	13.12	12.80
5	0.35	0.47	1 16	2 12	0.70	0.28	0.26
10	0 13	0.14	0 18	0 21	0.18	0 14	0.15
15	0.16	0 15	0 14	0.17	0.16	0.16	0.13
20	0 16	0.18	0 15	0.16	0.15	0 15	0.15
25	0.17	0 16	0 16	0.17	0 15	0 16	0.15
30	0 13	0.13	0.13	0 13	0 13	0 12	0.13
Total	7.56	13 88	18.32	29.21	13 00	14 13	13.77

* In these leachings, the soil was not removed from the paper on which it was dried; in the other leachings the soil was removed from the paper.

the three soils were thoroughly leached and placed in the papers in a drying oven kept at 50°C. After different intervals, the soils were removed from the papers, thoroughly extracted with water, and filtered through the same

papers.   The filtrate was clarified by the addition of some powdered CaO and $CuSO_4$ and again filtered.   The nitrate determinations in this experiment, as well as in the previous experiments, were made by means of the phenol-disulfonic acid method.   After the first 15 days, in which six leachings were made, all determinations were accompanied by control determinations, where only the water and reagents were employed.   Only the averages are reported, as there was usually very little variation between the duplicates of the controls or among the quadruplicates of the soils.   All the results are calculated on the basis of 100 gm. of soil.

The results presented in table 12 point to three highly important conclusions; namely, (1) that the water and reagents used contain about as much nitrate

TABLE 12

Influence of soil treatment on the amount of nitrate leached from soil after varying periods of drying at 50°C.

Milligrams of NO_3-N in 100 gm. of soil

DRYING PERIOD	SOIL 5A*	SOIL 11A*	SOIL 18B*	CONTROL†
days				
0	0.700	0.230	11.370	.
2	0.333	0.067	1.267	
4	0.130	0.060	0.153	
7	0.167	0.093	0.133	.
11	0.093	0.077	0.080	
15	0.157	0.093	0.090	.
19	0.110	0.077	0.073	0.073
23	0.077	0 083	0.077	0.060
28	0.077	0.063	0.067	0.053
35	0.107	0.097	0 097	0.073
42	0.097	0.093	0.093	0.087

* The following amounts of ammonia were found in 100 gm of soil, at time of sampling: 5A—0.7 mgm., 11A—16.4 mgm., 18B—0 9 mgm.

† Distilled water + reagents.

as that found in the soil after the first few leachings; (2) that soil 11A, in which only little nitrate was produced under natural conditions, gave, after the first leaching of the soil before drying, approximately the same amount of nitrate as the control; (3) only those soils (5A and 18B), in which considerable nitrate was present originally, gave any significant amounts of nitrate after the first leaching, as a result of drying.

DISCUSSION

The biological nature of the process of nitrate formation in soil has been recently questioned.   The fact that ultra-violet radiations are able to oxidize ammonia in salt solutions to nitrite was suggested as an explanation for the probable rôle of sunlight in the formation of nitrate in soil.   On the other

hand, the fact that when soil is dried at 50–60°C., small amounts of nitrates can be repeatedly extracted by leaching with water has been suggested as an explanation for the formation of nitrate in soil by physicochemical processes of heat and drying.

In all these investigations, the main facts were overlooked; namely, (1) before nitrate is formed in the soil, ammonia must be produced, and, (2) before ammonia is produced in the soil, organic nitrogen must be present, since the ammonia is either added to the soil or is liberated as a result of decomposition of the organic matter added to or present in the soil. The formation of nitrate is only the final step of a series of transformations which nitrogen undergoes in the soil. Although ultra-violet radiations unquestionably can bring about the oxidation of ammonia to nitrite, they also bring about the reduction of nitrate to nitrite, the latter being the stable form into which the nitrogen is transformed by radiations. Further, these processes we found to take place largely in solutions. It is a far-fetched conclusion that these observations also apply to the formation of nitrate in normal soils. One need not deny, of course, the fact that under very bright sunlight, small amounts of nitrite may be formed photochemically in soil. The formation of nitrate, however, as a result of these reactions, still remains to be established.

As to the effect of higher temperatures and of drying of the soil upon the formation of nitrate, here as well certain observations are misinterpreted to explain important and universal soil processes. When a soil is leached with water to remove the nitrate, the fact that no further nitrate can be immediately extracted is no proof that all the nitrate has been removed. A number of investigators, beginning with Warington in 1882, have amply established the fact that a great many leachings are necessary to remove all the nitrate from the soil. Further, it has also been definitely established that drying of soil favors the removal of the nitrate; consequently, a soil repeatedly dried and wetted will liberate much more nitrate than a soil kept continuously wet. The exact mechanism of this process is still to be established; it may be due either to the difficulty of diffusion of the residual nitrate from the soil film into the free water, as suggested by King (12), or to the presence of various intermediary oxidation products which are completely oxidized to nitrate on drying of soil, as suggested by Fischer (8).

The investigations reported in this paper do not support either the photochemical oxidation claim or the physiochemical claims, namely, that the formation of nitrate in soil is due predominantly either to chemical action of sunlight or to the periodic drying of soil rather than to the action of biological agents. To be sure, the light effects in New Jersey are different from those in India. At best, however, the chemical processes can account for only a small part of the total nitrate produced in the soil. Bacteria must, therefore, still be considered as playing the predominent rôle in the process of nitrification under normal soil conditions.

SUMMARY

A study of the effect of sunlight upon the oxidation of inorganic and organic forms of nitrogen to nitrate in soil brought out the following results:

When a sandy soil was used in an air-dry condition, no nitrification took place, even when the soil was exposed to full sunlight for a period of 470 hours. In a loam soil, no nitrification of the soil's own nitrogen or of added organic nitrogen occurred, but ammonium sulfate added to the soil was actively oxidized; a slight difference in favor of the soil exposed to sunlight cannot be considered of sufficient significance to be ascribed to the effect of photonitrification.

When the soils were kept in a moist state, either in pots or in large glass dishes, no significant difference could be detected in the rate of nitrification of different forms of nitrogen between the covered and the exposed soils, either in the light sandy soil or in the heavy loam soil.

A temperature of 27–37°C. was found to be most favorable for the activities of the nitrifying bacteria. Above and below that range of temperatures, the rate of nitrification was reduced.

The results thus obtained cannot support the claim that photonitrification plays an important part in normal soil processes, especially in soils of temperate regions.

A study was also made of the effect of heating and drying of soil upon the formation of nitrate, with the following results:

Drying of leached soil at 50° and 65°C., followed by leaching, yielded small amounts of nitrate.

Added sources of nitrogen in the form of ammonium salt and organic substances did not result in any increase in the amount of nitrate obtained by drying the soils.

Nitrate added to the soil was not recovered in the first leaching; but small amounts were obtained after several dryings and leachings.

As a result of biological oxidation of ammonia, the nitrate was not removed completely from the moist soil; on drying the soil and extracting with water more nitrate was obtained.

The water used for leaching the soil and the reagents employed in the determination gave almost as much nitrate as the soil, after the first few leachings; in a soil with little biological nitrification, the nitrate found after the first leaching was always about the same as that found in the control.

These results lead to the only possible conclusion that the nitrate extracted from the soil by leaching with water, after the soil has been dried, is not produced as a result of drying but has been rendered more readily extractable as a result of the process of drying.

The final conclusion from these two groups of studies on the formation of nitrate in soil is that the biological oxidation of ammonia to nitrite and of nitrite to nitrate must still be considered as the all-important process in the formation of nitrate in soil.

REFERENCES

(1) ALLEN, K. R., AND BONAZZI, A. 1915 On nitrification. Ohio Agr. Exp. Sta. Tech. Bul. 7.

(2) BAUDISH, O. 1911 Über Nitrat- und Nitrit- Assimilation. *Ber. Deut. Chem. Gesell.* 44: 1009–1013.

(3) BERTHELOT, D., AND GAUDECHON, H. 1911 La nitrification par les rayons ultra-violets. *Compt. Rend. Acad. Sci.* [Paris]. 152: 522-524.

(4) CAREY, C. L., AND WAKSMAN, S. A. Oxidation and reduction of nitrogenous compounds in sea water. Unpublished data.

(5) CORBET, A. S. 1934 The formation of hyponitrous acid as an intermediate compound in the biological or photochemical oxidation of ammonia to nitrous acid. I. Chemical reactions. *Biochem. Jour.* 28: 1575-1582.

(6) DHAR, N. R. 1935 Influence of Light on Some Biochemical Processes. Soc. Biol. Chem. (Indian Inst. Sci.), Bangalore.

(7) DESAI, S. V., AND FAZAL-UD-DIN 1936 The nature of nitrification in soil. *Indian Jour. Agr. Sci.* 6· 777-783.

(8) FISCHER, H. 1913 Vom Trocknen des Bodens. *Centbl. Bakt.* (II) 36: 346-349.

(9) FRAPS, C S., AND STERGES, A. J. 1935 Effect of sunlight on the nitrification of ammonium salts in soils. *Soil Sci.* 39· 85-94.

(10) GUSTAFSON, A. F. 1922 The effect of drying soil on the water-soluble constituents. *Soil Sci.* 13 173-213.

(11) KHALIL, F. 1929 The effect of drying on the microbiological processes in soils. *Centbl. Bakt* (II), 79 93-107.

(12) KING, F. H. 1908 The suspension of solids in fluids and the nature of colloids and solutions. *Trans. Wis. Acad. Sci.* 16. 275-288 [Abs. in *Exp. Sta. Rec.* 21· 19-20 (1909)].

(13) LEATHER, J. W. 1911-12 Records of drainage in India. *India Dept. Agr. Mem.,* *Chem. Ser* 2 63-140.

(14) MOORE, B. 1917 The formation of nitrites from nitrates in aqueous solution by the action of sunlight and the assimilation of the nitrites by green leaves in sunlight. *Nature* [London] 50: 338

(15) OSUGI, S., AND AOKI, M 1936 On the photooxidation of ammonium compounds in solution and soil *Jour Sci Soil Manure* [Japan] 10: 11-24.

(16) RAKESTRAW, N. W, AND HOLLAENDER, A. 1936 Photochemical oxidation of ammonia in sea water. *Science* 84: 842-843.

(17) ROSSI, G. 1933, 1935 La nitrification dans le terrain par simple action physico-chimique. *Bol. Soc Ital Soc Internatl. Microbiol.* 5: 132-136 (1933); *VI. Cong. Internatl. Bot., Sect. Mycol , Bact. et Agron* 2. 174-176 (1935).

(18) RUSSELL, E. J., AND HUTCHINSON, H. B 1909, 1913 The effect of partial sterilization on the production of plant food. II. The limitation of bacterial numbers in normal soils and its consequences. *Jour. Agr Sci.* 3: 111-144 (1909); 5: 152-221 (1913).

(19) STEVENS, K. R., AND WITHERS, W. A. 1910 Studies in soil bacteriology. III. Concerning methods of determination of nitrifying and ammonifying powers. *Centbl. Bakt* (II) 25. 64-80.

(20) THIELE, H 1906, 1907 Einige Reaktionen im ultravioletten Lichte. *Arch Hyg.* 57· 47 (1906); *Ber. Deut. Chem. Gesell.* 40. 4914-4916 (1907).

(21) WAKSMAN, S. A , AND STARKEY, R. L. 1923 Partial sterilization of soil, microbiological activities and soil fertility. *Soil Sci.* 16: 137-157, 247-268, 343-357.

(22) WARBURG, E. 1918 Über den Energieumsetz bei photochemischen Vorgängen. VIII. Die Photolyse waszriger Lösungen und das photochemische Aquivalent-gesetz. *Sitzber. Preuss. Akad. Wiss.* 1918. 1228-1246.

(23) WARINGTON, R. 1882 The determination of nitric acid in soils. *Jour. Chem. Soc. Trans.* 41: 351-360.

(24) ZOBELL, C. E. 1935 Photochemical nitrification in sea water. *Science* 77: 27-28.

(25) ZOLCINSKI, J. 1924 Sunlight and chemical nitrification. *Rocz. Nauk Rolnicz.* 10: 310-332; *Internatl. Rev. Sci. and Pract. Agr.* [Rome] 2: 838; *Pedology* 19: 80-97.

EVAPORATING THE WATER WITH BURNING ALCOHOL AS A RAPID MEANS OF DETERMINING MOISTURE CONTENT OF SOILS[1]

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It will be generally admitted that a need exists for a rapid, dependable method for determining the moisture content of soils, especially under field conditions.

PRINCIPLE OF NEW METHOD

The facts that alcohol is capable of extracting the moisture from soils (2, 5), that it burns, and that upon burning it also tends to evaporate the water dissolved in it, suggested the idea that the moisture content of soils might be determined by treating the soil with an excess of alcohol and then burning the alcohol.

The principle is to extract the soil moisture with alcohol and then, by burning the alcohol, to evaporate the water and at the same time dry the soil completely by the heat produced. By repeating the treatment and weighing the soil sample after each burning, complete drying is definitely assured. Methyl, ethyl, and propyl alcohols may be used. All three will ignite and burn when they contain as much as 40-45 per cent water by volume, although not all the water in these dilutions is evaporated.

PROCEDURE OF METHOD

The following materials are used for determining the moisture in soils by evaporating it with burning alcohol:

1. A metal cup 2 inches in diameter, 1 inch in height, with perforated bottom, and standing on four narrow legs $\frac{1}{4}$ inch high (pl. 1).[2]

2. A metal cup like the top of a Mason jar, about 3 inches in diameter, about 1 inch in height, and having a solid metal bottom.

3. A pair of ordinary crucible tongs.

4. Filter paper to fit the perforated bottom of cup 1.

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[2] The special cup can be obtained from The Wood and Metal Products Co , Box 234, Bloomfield Hills, Michigan A high-grade triple beam balance used in connection with this method is that represented in catalog no. 1966, of Arthur Thomas & Company.

5. *A very high-grade triple beam balance, sensitive to about* 0.01 gm.[2]
6. Methyl alcohol.

The procedure for making a moisture determination consists first of laying a filter paper in the bottom of cup 1, placing this cup in cup 2, and weighing them together. During the entire procedure the two cups are always kept one inside the other and are always weighed together as a single unit. A layer of soil about ½ inch thick, amounting to about 20–25 gm., is placed in cup 1, and the cups are weighed again. On the top of the soil layer is then poured gradually about 25 cc. of methyl alcohol, and after most of it has drained through the soil, it is ignited. After all the alcohol is burned and the flame goes out the cups are weighed. In order to be certain that all the water in the soil is expelled, 10–15 cc. alcohol is poured into cup 2 and burned, —and the cups are weighed again. This procedure of burning small quantities of alcohol in cup 2 and weighing the cups is repeated until the weight becomes constant. It was experimentally found that the moisture content of a soil can be determined more rapidly and with considerably less consumption of alcohol by adding a small amount of alcohol at a time than by adding a very large amount at the outset.

To burn 15 cc. of pure methyl alcohol in cup 2 takes about 3 minutes. To evaporate 5 cc. of water in cup 2 requires 10 minutes and 30 cc. alcohol, added in two 15-cc. portions. The time required to make a soil moisture determination by this method depends upon the amount of water present in the soil, and upon the extent to which the alcohol extracts the water as it percolates through the soil. It is quicker to evaporate the water when it is dissolved in alcohol than when it is in the soil. To determine the moisture content of soils that are easily permeable, such as sands or even clays in the granular condition, and of low moisture content takes about 5 to 15 minutes including the time for weighing. Soils that have a very high moisture content, especially if they are impermeable such as heavy puddled clays, require from 10 to 35 minutes for a moisture determination. On the other hand, work may be carried forward on a number of soils at the same time, as the procedure does not require constant attention.

Toward the end of the drying when all the water has been driven off, the soils attain a temperature of about 130°C. near the center of the cup and about 160°C. near the walls of the cup.

EXPERIMENTAL

From fundamental considerations it is reasonable to expect that the water in soils could be expelled by means of burning alcohol. The only question is whether the treatment is so drastic as to burn also the organic matter present and to drive off the combined water from the mineral portion of the soil. In subjecting the method, therefore, to a critical and comprehensive examination, many different soils having different organic matter and combined water contents were used. In table 1 is shown a comparison of the moisture contents of these soils as obtained by the burning alcohol method and by the

standard oven method wherein the soils were dried for 24 hours at a temperature of 110°C. In the same table are shown the percentage of organic matter

TABLE 1

Comparison between the alcohol burning method and the standard oven-drying method for determining moisture content of soils

SOILS	DEPTH	MOISTURE CONTENT		COMBINED WATER	ORGANIC MATTER
		Alcohol method	Oven method		
	inches	*per cent*	*per cent*	*per cent*	*per cent*
Silica gel		0.68	0 70	4.37	0
Fuller's earth		8 49	8.35	9 53	0
Bentonite		12 61	12 68	4 88	0
Hagerstown clay loam	0–6	4 92	5.05	8 20	0
Hagerstown clay loam	70–80	4 38	4.25	8.00	0
Irredel loam	12–16	5 60	5 38	8 31	0
Bladen loam	12-18	3 10	3 31	5.30	0
Decatur clay	0–6	1 32	1.25	4 04	0
Davidson clay loam	24 30	2.20	2 18	8.46	0
Calcareous clay	C horizon	3 70	3 92	3.61	0
Susquehanna clay	2–6	3 75	3 98	4 48	0
Lufkin clay	10-16	10.11	10.43	5.90	0
Mavon silt loam	Surface	1 95	2 03	2 94	2 44
Putman silt loam	Surface	2 35	2 48	2 99	3 82
Grundy silt loam	Surface	2 81	2 76	4 10	2.56
Bumer silty clay	Surface	3 75	3 96	3 79	3 43
Colby silt loam	Surface	2.56	2 38	3 12	3 52
Houston clay	A horizon	7 00	7 20	7 21	8 87
Fargo clay adobe	Surface	8.18	7 80	6 18	5 82
Fargo clay loam	6-16	5 20	5 35	4 06	4 38
Yolo silt loam	1-18	4 38	4 20	5 27	4 65
Lake Clark's clay	Surface	4.55	4 35	3 27	2 34
Webster silty clay loam	Surface	4 68	4 46	4 32	8 84
McKenzie clay	0–1	5 35	5 09	3 92	2 82
McKenzie clay	A horizon	7 53	7.28	5 29	4 43
Black clay adobe		5 25	5 42	7 79	0 80
Kerwin fine sandy loam	9–12	4 22	4 05	6 83	0 54
Stockton clay adobe	0–16	8.55	8 32	6 71	0 40
Clyde clay	Surface	6.40	6 55	5 20	10 32
Clyde soil		6 55	6 30	5.70	10 74
Organic mineral soil		8 9	5 89	4 98	12 18
Field soil no. 1		13 4	13 4		
Field soil no. 2		53 1	54 0		
Field soil no 3		14 08	13 58		
Field soil no. 4		18 35	19 00		
Field soil no. 5		26.30	25 90		
Field soil no. 6..		20 08	20 51		
Field soil no 7		37.18	38 06		
Field soil no 8		29 38	29 97		

and the percentage of combined water contained in many of these soils as determined by the distillation method (3). For the purpose of establishing

the principles under consideration, soils in the air-dry condition were preferred. In the air-dry condition soils permit of a more accurate sampling than do wet soils, especially the sticky types. The wet soils studied were mostly taken from the field, and, wherever possible, they were passed through a 2-mm. mesh sieve and thoroughly mixed. In the puddled condition, they were thoroughly kneaded to obtain uniformity in water content.

The experimental data presented in table 1 show: (a) The burning-alcohol method is as accurate for determining the moisture content of mineral soils as is the oven-drying method; the results by both methods agree remarkably closely. (b) The combined water of the mineral soils investigated was not influenced by the alcohol-burning procedure. (c) Organic matter up to about 11 per cent in mineral soils seemed to be unaffected by the burning alcohol. It is seen from the data that mineral soils containing up to about 11 per cent of organic matter gave off as much water or suffered the same loss in weight with the burning-alcohol method as with the oven-drying method. It is only in mucks and peats and in mineral soils containing more than 11 per cent organic matter, especially in an undecomposed state, that repeated burning of alcohol affects the organic matter by bringing about either decomposition or ignition.

DISCUSSION

There is no question that the moisture content of mineral soils can be determined by partial extraction with alcohol followed by evaporation with burning alcohol. The procedure can be carried out rapidly. It can also be accurate, as the heating can be repeated until the weight becomes constant. The failure of the burning-alcohol procedure to affect the organic matter in mineral soils containing not more than about 11 per cent may be explained on the grounds that the soils are heated intermittently for only a few minutes; that the organic matter in these soils is so diluted by the mineral matter that no ignition takes place; and that, as the cup containing the soil does not stand high above the base, the soil is not subjected to the most intense heat of the flame and consequently the temperature of the soil mass does not go above 130–160°C. Unless ignition is induced at the surface which comes in contact with the flame, these temperatures are not high enough to cause much decomposition of organic matter, especially of the humus type (3, 4), neither are they high enough to break down the mineral portions of the soils and drive off the combined water (1, 3). On the other hand, these temperatures may be sufficiently high to expel the weakly held water of hydration of some materials. Normal propyl alcohol evaporates the water almost twice as rapidly as either methyl or ethyl, but it is more expensive.

This new rapid moisture method could be used in main laboratories and in field laboratories for determining moisture content of soils. It should be of particular value in phases of work in which it is desired to determine the moisture content immediately. It could also be used for rapid drying of materials,

such as marls, for rapid analysis.   Studies are under way to determine whether it could also be used as a practical field method.

SUMMARY

Partial extraction with alcohol followed by drying with burning alcohol is presented as a rapid and accurate method of determining the moisture content in soils.

The procedure consists of treating the soil with alcohol in a special apparatus and burning the alcohol, the heat from which evaporates the water and dries the soil completely.

This alcohol-burning procedure raises the temperature of the soil to only about 130–160°C.   It has no influence on the combined water and no effect on organic matter up to about 11 per cent in mineral soils.   In mucks, peats, and in mineral soils containing more than about 11 per cent organic matter, especially in the undecomposed state, the method is not applicable.

In mineral soils containing less than about 11 per cent organic matter the method is as accurate in determining moisture content as is the standard oven-drying method.

The method is rapid.   It can determine the moisture content of sands in 5 to 10 minutes, loams in 10 to 20 minutes, and clays in 10 to 35 minutes, depending on their content of water and their permeability to alcohol.

REFERENCES

(1) BAVER, L , AND HORNER, G M. 1933 Water content of soil colloids as related to their chemical composition. *Soil Sci.* 36. 329 -353.
(2) BOUYOUCOS, G. J. 1931 The alcohol method for determining moisture content on soils. *Soil Sci* 32· 173–178
(3) BOUYOUCOS, G. J. 1933 The distillation method for determining the combined water and organic matter in soils. *Soil Sci.* 36. 471–483.
(4) BOUYOUCOS, G. J. 1934 A method for determining the degree of decomposition that unknown decayed vegetable organic materials have already undergone in nature *Soil Sci.* 38 477–482.
(5) BOUYOUCOS, G. J. 1936 The state in which the hygroscopic moisture exists in soils as indicated by its determination with alcohol. *Soil Sci.* 41. 443–447

PLATE 1

APPARATUS FOR DETERMINING MOISTURE CONTENT OF SOILS BY THE ALCOHOL-BURNING
METHOD

PLATE 1

DETERMINING SOIL MOISTURE CONTENT
GEORGE JOHN BOUYOUCOS
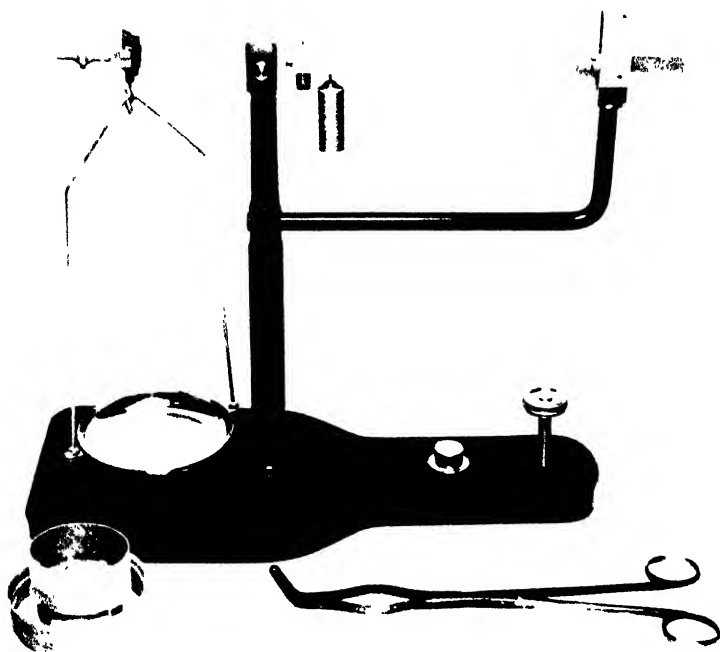
CELLULOSE DECOMPOSITION IN SYNTHETIC AND NATURAL SOILS[1]

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INTRODUCTION

Since Mitcherlich's observation in 1850 that decomposition of cellulose is brought about by biological agents, a vast amount of work has been done on this subject. At first, bacteria (anaerobic, aerobic, thermophilic, and denitrifying) were considered to be the only agents active in the decomposition process, but it was soon found that the filamentous fungi, actinomyces, fleshy fungi, and even invertebrates were also able to decompose cellulose. Filamentous fungi were believed, until comparatively recently, to be active principally in acid soils, but Waksman (17, 18), Rege (12), and Norman (11) showed that they were of great significance even in normal soils neutral or even slightly alkaline in reaction.

The rate of decomposition of cellulose and of other plant constituents was found to depend on the chemical and mechanical nature of the soil, on the types of organisms present, on the nature and abundance of available nitrogen, on the soil reaction, temperature, moisture, and on other factors. The mechanical composition influences the degree of aeration, the moisture-holding capacity, and the nutrient-supplying power of the soil, and its chemical composition determines the amount and the nature of the available nutrients, particularly nitrogen and phosphates. Different groups of organisms have varying capacities for decomposing cellulose, as is conclusively shown by Waksman (15). Some organisms decompose cellulose only if other more easily available organic sources are absent, whereas other organisms, like *Cytophaga hutchinsonii*, utilize only cellulose as a source of energy. Some organisms possess only a facultative specificity, i.e., they prefer cellulose as a source of energy but are also able to utilize other organic compounds. Winogradsky (20) and Waksman and Starkey (18) have shown, however, that in general the addition of cellulose to soil encourages the growth of fungi, whereas proteins and soluble carbohydrates stimulate the growth of bacteria and actinomyces. It is generally believed that, except for the thermophilic or strictly anaerobic organisms, inorganic sources of nitrogen are preferred to organic sources. It

has been shown by Heukelekian and Waksman (7) that, on an average, 1 part of nitrogen is required for every 30 parts of cellulose decomposed in pure cultures of fungi. In impure cultures, however, they found the ratio to be much wider, about 1:50.

Bertrand and Compton (2) found 46°C. to be the optimum temperature for the activity of cellobiase. Jensen (8), however, in his recent studies with Australian soils, found that the maximum activity of the cellulose-decomposing fungi was at 15°C.

As Norman (11) stated, it is not necessary to assume that one organism alone is responsible, under natural conditions, for the complete breakdown of the cellulose molecule to its ultimate products, CO_2 and water; on the contrary it is probable that much of the decomposition proceeds by stages, being affected by a whole series of organisms, each attacking the particular degradation product that most suitably meets its energy requirements. Rege (12) showed, however, that under certain conditions a pure culture of a fungus could decompose cellulose as rapidly as the mixed soil flora.

The associative growth of two or more organisms may be competitive or coöperative, as shown by Norman (11) and more recently by Waksman and Hutchings (16). These investigators found that Trichoderma, a strong cellulose-decomposing organism, in pure culture attacked the proteins of alfalfa plants in preference to cellulose, but when preceded by Rhizopus, a non-cellulose-utilizing organism, it readily attacked cellulose. They also found that the presence of non-cellulose-decomposing fungi and bacteria hastened cellulose decomposition by organisms capable of effecting such decomposition.

The influence of reaction on decomposition of soil organic matter has received considerable attention. Charpentier (3) and Barthel and Bengtsson (1) found that liming has no beneficial effect on cellulose decomposition as a whole and that it encourages only certain groups of organisms concerned in the process. This has been further borne out by the work of Karnicka and Ziemiecka (9), who found that addition of lime to an acid soil does not greatly improve its cellulose-decomposition power.

In the present investigation an attempt has been made to standardize the conditions that affect the decomposition of cellulose in soil. A study has also been made of the various groups of microörganisms developing under different conditions. This has been correlated with the actual cellulose decomposition brought about by these organisms. Both natural soil and synthetic soil, consisting of sand and bentonite, were used in these investigations. The synthetic soil described previously (10) was found to be of great assistance because it resembled in mechanical composition a light loam and yet lacked the complexity that hinders proper interpretation of results obtained from use of natural soils.

EXPERIMENTAL

Cellulose was estimated by hydrolysis with strong sulfuric acid (19). The numbers of fungi were determined by plating on Waksman's acid agar, and

those of bacteria and actinomyces, by use of albumen agar. The numbers of cellulose-destroying bacteria were determined by the dilution method as modified by Dubos (6). Use was made of the Conn direct-count method (5) and the Rossi-Cholodny contact-slide method (4, 13), for following the changes in the microflora active in the decomposition of cellulose.

TABLE 1

*Influence of reaction and nitrogen source upon the development of different microorganisms in the process of cellulose decomposition**

NITROGEN SOURCE†	INITIAL REACTION	10 DAYS' INCUBATION			30 DAYS' INCUBATION		
		Fungi	Bacteria	Cellulose-decomposing bacteria	Fungi	Bacteria	Cellulose-decomposing bacteria
	pH						
$(NH_4)_2SO_4$	9.5	0	59.7	10	2.3	6.7	1,000
$(NH_4)_2SO_4$	7.5	0	86.0	1,000	75.0	3.9	1,000
$(NH_4)_2SO_4$	5.5	130	35 0	10	750 0	2 0	10
$(NH_4)_2SO_4$	3 5	170	24 6	10	231.5	0.5	1
$NaNO_3$	9 5	0	49 3	10	4 5	31 5	1,000
$NaNO_3$	7 5	5	49 2	100	73.5	42 0	1,000
$NaNO_3$	5 5	200	11 9	10	585 0	110.5	100
$NaNO_3$	3 5	125	9 5	10	560 0	1.0	10

* Counts of fungi and cellulose-decomposing bacteria in thousands per gram, bacteria developing on plate in millions per gram. All figures represent the average of duplicates.

† $(NH_4)_2SO_4$ at the rate of 20 8 mgm N per 100 gm. medium; $NaNO_3$ at the rate of 19.0 mgm N per 100 gm. medium.

Effect of reaction and of nitrogen source on cellulose decomposition

The synthetic soil described elsewhere (10), namely, 97.5 per cent sand + 2.5 per cent bentonite, was adjusted to different reactions with dilute hydrochloric acid, as follows:

AMOUNT OF 0 2834 N HCl ADDED	REACTION
cc.	*pH*
0	9.5
8 0	7.5
12.0	5.5
16.0	3.5

One per cent ground filter paper containing 768.74 mgm. pure cellulose was added to each mixture. One set of containers received ammonium sulfate at the rate of 20.8 mgm. nitrogen per 100 gm. as a source of nitrogen, and a second set received sodium nitrate at the rate of 19.0 mgm. nitrogen per 100 gm. One cubic centimeter of a fresh soil suspension was added as inoculum. The numbers of fungi, of bacteria developing on the plate, and of cellulose-destroying organisms were determined after intervals of 10 and 30 days (table 1).

Contact slides were prepared after an incubation period of 18 days, and the

types of organisms developing in the different treatments were recorded. Determinations of the amount of cellulose decomposed and the corresponding amounts of ammonia and nitrate nitrogen consumed were also made (table 2). The results of this experiment can be summarized as follows:

From the point of view of cellulose decomposition, an initial reaction of pH 5.5, with sodium nitrate as a source of nitrogen, seems to be most suitable. The initially acid reaction encouraged the growth of fungi, but a later change in reaction to slight alkalinity encouraged bacterial development.

The numbers of cellulose destroyers in the initial stages show no correlation with actual cellulose decomposition.

A rapid loss of ammonia nitrogen at an alkaline reaction was found to be due to the transformation of the ammonia to nitrite and nitrate.

TABLE 2

Influence of reaction and nitrogen source upon cellulose decomposition*

(All figures represent the averages of duplicates)

NITROGEN SOURCE†	INITIAL REACTION	10 DAYS' INCUBATION		30 DAYS' INCUBATION		RATIO CELLULOSE NITROGEN	FINAL REACTION
		Cellulose decomposed	Nitrogen consumed	Cellulose decomposed	Nitrogen consumed		
	pH	*mgm.*	*mgm*	*mgm*	*mgm.*		pH
$(NH_4)_2SO_4$	9.5	18.8	2.8	125.7	12.5	10.0	5 9
$(NH_4)_2SO_4$	7.5	18.8	2.1	153 8	6.75	23.0	5.3
$(NH_4)_2SO_4$	5.5	69.4	2.25	135.0	4.5	30.0	4.4
$(NH_4)_2SO_4$	3.5	41.3	0	78.8	0		4.1
$NaNO_3$	9.5	18.8	1 3	48 8	1.25	39 0	9.3
$NaNO_3$	7.5	60.0	5 3	172 5	5.8	29 7	8 2
$NaNO_3$	5.5	18.8	5.85	286 5	9.2	31.1	7.4
$NaNO_3$	3.5	18.8	5.15	106.9	5.0	21.4	4 9

* Medium consisting of 97.5 per cent sand + 2.5 per cent bentonite Initial cellulose concentration = 768.74 mgm. per 100 gm.

† $(NH_4)_2SO_4$ at the rate of 20.8 mgm. N per 100 gm. medium; $NaNO_3$ at the rate of 19 mgm. N per 100 gm. medium.

A large number of cellulose-destroying bacteria were found after 10 days' incubation at the more alkaline reactions with ammonium sulfate as a source of nitrogen, although the decomposition of cellulose was much less than that at the more acid reactions. The total bacterial count after 30 days' incubation with ammonium sulfate as a source of nitrogen was much lower than at the corresponding reactions with sodium nitrate as a source of nitrogen.

The numbers of fungi were consistently higher at acid reactions than at alkaline reactions, and the total numbers of bacteria and the numbers of cellulose destroyers were lower.

Contact slides prepared from the different tumblers after 10 days according to Conn's modification of the Cholodny method showed very good correlation with the types of organisms obtained by the plate method. Rod-shaped bacteria, cocci, and actinomyces were the most prominent organisms on the slides prepared from the alkaline media, and fungus hyphae were most prominent on the slides prepared from the acid media.

The ratio of cellulose decomposed to nitrogen utilized varied, in most cases, between 25:1 and 35:1. The ratio, which was found to be about 10, in the most alkaline medium

with $(NH_4)_2SO_4$ as a source of nitrogen must be corrected for the ammonia nitrogen that was converted into nitrite and nitrate.

Most of the nitrogen utilized by the organisms was later recovered in a soluble form, apparently because of the mineralization of the microbial cell substance.

Effect of temperature on cellulose decomposition

Synthetic soil medium containing 1 per cent cellulose in the form of ground filter paper was adjusted to pH 5.5, which was previously found to be best suited for cellulose decomposition. Nitrogen was supplied in the form of sodium nitrate, and the inoculum was added in the form of 1 cc. of the soil suspension. To each tumbler 200 mgm. potassium phosphate was added.

TABLE 3

Effect of temperature on the development of different microorganisms in the process of cellulose decomposition*

TEMPERATURE OF INCUBATION	14 DAYS' INCUBATION		28 DAYS' INCUBATION			42 DAYS' INCUBATION			56 DAYS' INCUBATION			70 DAYS' INCUBATION		
	Fungi	Bacteria	Fungi	Bacteria	Cellulose decomposing bacteria	Fungi	Bacteria	Cellulose decomposing bacteria	Fungi	Bacteria	Cellulose decomposing bacteria	Fungi	Bacteria	Cellulose decomposing bacteria
°C.														
7	†		45 0	0 155	0 1				185	18 5	1	2950	259.5	10.0
20			950 0	93.5	1000 0				750	178 0	1000	1800	253 0	10,000 0
27	1200	12 5	1200 0	194 0	1000 0	550	160 0	1000				950	302 0	1000 0
37	1485	54 0	2150 0	87.0	1000 0	1550	103.5	1000				1900	86 0	1000 0
55	few	few	2 9	0.31	1 0					0 5	1	few	0.14	0.1

* $NaNO_3$ used as the source of N. Initial reaction pH 5 5. Numbers of organisms reported as thousands per gram for fungi and cellulose-decomposing bacteria, and millions per gram for bacteria developing on plate.

† Not determined.

Two tumblers were incubated at each of the following temperatures: 7°C., 20°C., 27°C., 37°C., and 55°C. The numbers of bacteria, fungi, and cellulose destroyers, as well as the amount of cellulose decomposed and the nitrogen utilized, were determined at various intervals. The results, presented in tables 3 and 4, warrant the following conclusions:

Cellulose decomposition was at first most active at 37°C., but after about 8 weeks, decomposition at 20° and at 27°C., had become as active as at the higher temperature. The ratio of cellulose decomposed to nitrogen utilized was found to be 40:1 in the case of maximum decomposition.

The numbers of fungi were highest at 37°C. for 8 weeks, but after 10 weeks the numbers were highest at 7°C.

The bacterial counts were highest at 27°C. throughout the experiment after the first 2-week period, during which the tumblers incubated at 37°C. gave the higher counts.

The numbers of cellulose destroyers were practically the same at 20°, 27°, and 37°C., except during the last period of incubation.

Ammonia was determined at the end of 10 weeks and was found in concentrations of 1.5 to 2.0 mgm. at 20°, 27°, and 37°C., pointing to the slow decomposition of the microbial cell substance.

The fungi developing at different temperatures were found to consist of species of yeasts: Monilia and Humicola predominated at 6–8°C., Trichoderma predominated at 27°C., and various species of Penicillium and Aspergillus were most active at 37°C.

TABLE 4

Effect of temperature on cellulose decomposition

TEMPER-ATURE OF INCU-BATION	14 DAYS' INCUBATION		28 DAYS' INCUBATION		42 DAYS' INCUBATION		56 DAYS' INCUBATION		70 DAYS' INCUBATION		RATIO CELLULOSE NITROGEN
	Cellulose decomposed	Nitrogen consumed	Cellulose decomposed	Nitrogen consumed	Cellulose decomposed	Nitrogen consumed	Cellulose decomposed	Nitrogen consumed	Cellulose decomposed	Nitrogen consumed	
°C.	mgm	mgm	mgm.	mgm	mgm	mgm	mgm	mgm.	mgm.	mgm	
7	...	...	147.9	0.1			245.0	8.0	329.3	7.8	43
20	...		413.6	13 0			760.5	19.0	777.4	19.0	41
27	231.8	7.2	393.0	12.4	704.3	19.0			741.8	19.0	39
37	330.3	9.7	546.8	13.9	741.8	17.8			760.5	19.0	40
55	166.1	.	136.1	1 2			203.4	4.3	194.6	3 8	51

Cellulose decomposition as related to types of organisms developing in different soils

In order to determine the influence of continuous fertilizer treatment upon the development of various groups of microörganisms utilizing cellulose as a source of energy, four samples of soil were obtained from the permanent field plots of the New Jersey Agricultural Experiment Station. These plots had the following treatments for the last 25 years:

PLOT NUMBER	TREATMENT OF SOIL	REACTION	TOTAL NITROGEN
		pH	per cent
7A	No fertilizer or lime	4.5	0.058
7B	Received only 2 tons of limestone per acre every 5 years	6 3	0 065
18A	Yearly additions of 320 pounds superphosphate, 160 pounds muriate of potash, 4,000 pounds cow manure, and 320 pounds NaNO₃ per acre	6 0	0.140
18B	Same as 18A + lime at the rate of 2 tons per acre every 5 years	6.8	0.136

The soils while fresh were put through 3-mm. sieves, and the moisture content was determined. Three pots containing 800-gm. portions of soil were prepared from each of the four soils and treated as follows:

POT NUMBER	TREATMENT
1	No cellulose
2	10 gm. cellulose
3	10 gm. cellulose + 200 mgm. nitrogen
	as $NaNO_3$ + 0.5 gm. K_2HPO_4

A duplicate set of pots was prepared for soil 18B and exposed to the sun on clear days throughout the summer months. The soil was sampled with

TABLE 5

Influence of soil treatment on bacterial population in the process of cellulose decomposition

NATURE OF SOIL	TREATMENT OF SOIL	2 WEEKS' INCUBATION			4 WEEKS' INCUBATION			7 WEEKS' INCUBATION		
		Plate count	Direct count	Cellulose destroyers	Plate count	Direct count	Cellulose destroyers	Plate count	Direct count	Cellulose destroyers
7A	Untreated	2 0	630	0 in 1/100	1 0	210		2.0	157	0.1
7A	Cellulose	2.0	315	1	1 0	105	1	2.0	210	1.0
7A	Cellulose + nitrogen	29.0	630	10	92.0	210	100	101.0	336	1,000.0
7B	Untreated	6.0	241	1	4.0	105	10	3 0	157	1.0
7B	Cellulose	6.0	262	100	6 0	105	100	32 0	105	1,000 0
7B	Cellulose + nitrogen	121.0	472	100	114 0	367	1,000	129.0	630	10,000 0
18A	Untreated	17 0	315	1	16 0	157	100	12.0	367	1,000.0
18A	Cellulose	22.0	388	100	20.0	157	1,000	29 0	262	1,000.0
18A	Cellulose + nitrogen	80.0	577	100	100.0	840	1,000	A.†	997	1,000.0
18B	Untreated	14 0	262	1	21.0	105	100	13 0	273	1,000.0
18B	Cellulose	26.0	525	100	25 0	78	100	21 0	210	1,000.0
18B	Cellulose + nitrogen	90.0	840	100	99.0	577	1,000	70.0	525	1,000.0
18B	Untreated‡	12.0	147	1	23 0	315	10	A †	210	100.0
18B	Cellulose‡	18.0	210	100	21.0	315	100	A.†	157	100.0
18B	Cellulose‡ + nitrogen	47.0	157	100	61.0	157	1,000	10.0	157	10,000.0

* Numbers of bacteria determined by the plate and direct count method reported as millions per gram; cellulose destroyers reported as thousands per gram

† A. = Actinomyces.

‡ Exposed to the sun.

a sterilized cork borer, and determinations were made of the total number of bacteria by the plate and the direct count methods. Determinations of the total numbers of fungi and actinomyces of cellulose-destroying organisms for residual cellulose and nitrate were made as described previously. Contact slides were used to follow the changes in the microflora of the different soils at different stages of cellulose decomposition.

The results reported in tables 5 to 8 lead to the following conclusions:

As a result of continuous treatment the soils may become unfavorable for general micro-biological activities, but they still contain a population of organisms capable of decomposing cellulose.

The nitrate content of most of the soils increased after an incubation of 8 weeks (table 8).

When cellulose alone had been added to the soil, the nitrates produced in the soil were rapidly used up.

Sunlight had a slightly injurious effect on cellulose decomposition. The injurious effect, however, had a tendency to decrease with the lapse of time.

The numbers of fungi were greater in the unlimed soils than in the limed ones.

TABLE 6

*Specific microflora developing on plates from cellulose-treated soils**

NATURE OF SOIL	TREATMENT OF SOIL	2 WEEKS' INCUBATION			4 WEEKS' INCUBATION			7 WEEKS' INCUBATION		
		Fungi	Bacteria	Actino-myces	Fungi	Bacteria	Actino-myces	Fungi	Bacteria	Actino-myces
7A	Untreated	150	2		200	1	0.3	150	2	
7A	Cellulose	170	2	0.2	270	1	0.9	200	2	
7A	Cellulose + nitrogen	2,000	29		3,000	92		2,700	101	5.0
7B	Untreated	80	6	2.0	50	4	3.0	120	3	7.3
7B	Cellulose	90	6	1.6	90	6	3.3	100	32	
7B	Cellulose + nitrogen	700	121	17.0	1,400	114	22 0	1,900	129	38.0
18A	Untreated	200	17	3.0	260	16	4.0	190	12	16.0
18A	Cellulose	350	22	7.0	340	20	5.0	500	29	10.0
18A	Cellulose + nitrogen	1,900	80	30.0†	2,400	100	28.0†	2,100	A.‡	115.0
18B	Untreated	200	14	11.0	120	21	4.0	160	3	21.0
18B	Cellulose	240	26	7.0	130	25	4.0	160	21	11.0
18B	Cellulose + nitrogen	1,100	90	42.0	800	99	24.0†	650	70	70.0
18B	Untreated§	200	12	10.0	180	23		60	A.‡	19.0
18B	Cellulose§	210	18	9.0	240	21	6.0	310	A.‡	50.0
18B	Cellulose§ + nitrogen	800	47	31.0	1,100	61	21.0	1,110	10	85.0

* Fungi reported as thousands per gram; bacteria and actinomyces reported as millions per gram.

† Mostly pink colonies with whorls.

‡ Mostly actinomyces.

§ Exposed to the sun.

Bacterial counts were, however, higher in the limed soils than in the unlimed soils. The numbers in the comparative pots exposed to the sun were lower than those kept in the dark.

Actinomyces were rather prominent in all cases. They increased in abundance, as did the bacterial numbers, with longer periods of incubation and were stimulated by the presence of nitrates. They were found at a maximum in the manured soils.

The addition of nitrogen seemed to stimulate primarily the development of the fungi.

Most of the fungi developing in the different soils were species of Penicillium and Trichoderma. Observations made after incubation for 4 weeks revealed the presence of Chaetomium in some of the soils.

The numbers of bacteria determined by plate counts and those determined by direct counts showed no correlation.

TABLE 7

Cellulose decomposed and nitrogen consumed in differently treated soils

NATURE OF SOIL	TREATMENT	4 WEEKS' INCUBATION		7 WEEKS' INCUBATION		12 WEEKS' INCUBATION	
		Cellulose decomposed*	Nitrate N consumed	Cellulose decomposed	Nitrate N consumed	Cellulose decomposed	Nitrate N consumed
		mgm	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
7A	Untreated						
7A	Cellulose	198.5	0.9	309.2	1.3	333.0	1.8
7A	Cellulose + nitrogen	894.0	21.2	990.5	21.5	1,013.5	16.0
7B	Untreated						
7B	Cellulose	233.6	1.1	401.0	2.0	363.2	2.7
7B	Cellulose + nitrogen	883.8	18.6	999.5	20 0	975.2	17.1
18A	Untreated						
18A	Cellulose	193.0	2.4	507.6	4.3	589.1	6.1
18A	Cellulose + nitrogen	883 8	18.3	923.9	18.6	949.1	18.0
18B	Untreated						
18B	Cellulose	33.3	1.8	452.8	3.3	608.4	5.0
18B	Cellulose + nitrogen	892.8	19.9	917 6	19 9	988.7	16.3
18B†	Untreated						
18B†	Cellulose	6 3	3.0	388.8	5 0	648 5	5.2
18B†	Cellulose + nitrogen	586.8	13.0	874 8	16.9	979.2	13.9

* Above control.

† Exposed to the sun 100 hours at the end of 4 weeks, 280 hours at the end of 7 weeks, 430 hours at the end of 12 weeks

TABLE 8

Changes in nitrate nitrogen during cellulose decomposition in different soils

NATURE OF SOIL	TREATMENT	NO₃-N INITIALLY ADDED	NITRATE FOUND IN 100 GM OF SOIL AFTER INCUBATION OF				
			4 weeks	8 weeks	12 weeks	15 weeks	
						Ammonia	NO₃
			mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*
7A	Untreated	0	1.23	1.33	1.75	.	2.22
7A	Cellulose	0	0.35	Trace	Trace	.	..
7A	Cellulose + nitrate	23.0	3.03	2.85	8.69	1.2	12 30
7B	Untreated	0	1.43	2.0	2.74		3.12
7B	Cellulose	0	0.32	0	0	.	0
7B	Cellulose + nitrate	23.0	5 88	5 0	8 59	1.6	9.09
18A	Untreated	0	2.72	4.3	6 06		6.89
18A	Cellulose	0	0.35	0	0		
18A	Cellulose + nitrate	23 0	7.40	8.7	11.11	1.2	14.28
18B	Untreated	0	2.17	3.3	5.0		4.65
18B	Cellulose	0	0.34	0	Trace		..
18B	Cellulose + nitrate	23.0	5.28	6.45	11.7	1 6	14.28
18B*	Untreated	0	3.33	5.0	5 2	...	8.70
18B*	Cellulose	0	0.35	0	Trace		..
18B*	Cellulose + nitrate	23.0	13.30	11.11	14.28	1.6	12.90

* Exposed to the sun 100 hours at the end of 4 weeks, 280 hours at the end of 8 weeks, 430 hours at the end of 12 weeks, 545 hours at the end of 15 weeks.

The following observations were made in connection with the contact slides:

The addition of cellulose alone encouraged the growth of fungi and actinomyces, irrespective of the fertilizer treatment. This was quite in accord with the corresponding plate counts.

The addition of cellulose and nitrogen to the soil that had received no fertilizer treatment encouraged the growth of fungi to such an extent that the bacteria and actinomyces were not prominent on the slides prepared after 1 week's incubation (pl. 1, fig. 1); however, they appeared in large numbers after 3 weeks. After this period they were found in abundance surrounding the fungal hyphae that appeared to be disintegrating (pl. 1, fig. 2 and 3).

The microflora in soil 18B, exposed to the sun, was clearly at a lower level of activity than the microflora of the corresponding soil in the dark.

GENERAL DISCUSSION

The results obtained in the foregoing experiments define clearly the influence of various factors upon the process of cellulose decomposition in the soil.

In the first two experiments only synthetic soil was used. This soil has the advantage of providing a medium that is mechanically very similar to the natural soil and yet is less complex. The fact that an initial soil reaction of pH 5.5, with sodium nitrate as a source of nitrogen, gave the highest decomposition of cellulose in any given time seems to indicate that for rapid cellulose decomposition an attack by the fungi, followed later by bacteria, is most advantageous. The fungi were at first greatly stimulated, but later, with a change in reaction towards neutrality, the bacteria and the actinomyces became active.

The results of the other experiments, using the four differently treated soils, justify the conclusion that, under optimum conditions of decomposition, fungi are the first to become active in cellulose decomposition except at very alkaline reactions; the hyphal material of the fungi is later attacked by bacteria, and the decomposition process is completed. This phenomenon was first suggested by Waksman and Skinner (17).

Actinomyces also play an important part in the decomposition of cellulose, as borne out by the fact that they are present in large numbers where decomposition is at all significant. It may be mentioned in this connection, however, that at low temperatures large numbers of yeast and Monilia appear without appreciable decomposition of the cellulose; also there were high fungal counts at a reaction of pH 3.6 without corresponding decomposition of the cellulose. This suggests the conclusion that although the fungi attack cellulose in the initial stages, the completion of the process is greatly favored by the association of bacteria.

Heukelekian and Waksman (7), working with pure cultures of Trichoderma and Penicillium, proved that fungi are capable of bringing about almost complete decomposition of cellulose. Recently Vartiovaara (14), working with *Sporotrichum carnis* and *Monotospora daleae*, two soil fungi, found that they were capable of bringing about almost complete breakdown of the cellulose in about 3 months.

The results of these investigations also bring out the fact that the initial activity of fungi hastens decomposition of cellulose. This would lead one to the obvious conclusion that for cellulose decomposition an initial acid reaction is most favorable. Sodium nitrate, urea, or calcium cyanamide, used as a source of nitrogen with an initial acid reaction of the soil or compost, permits a change in reaction to less acid, which would thus be ideal from a theoretical viewpoint.

It is further of interest to determine mineralization of the nitrogen, brought about by the disintegration of the microbial cells. The speed of this process varies with the type of organisms, as shown by Vartiovaara. He found that the cell substance of Sporotrichum and Monotospora mineralized to the extent of only 16 per cent, whereas 41 per cent of the cell substance of *Aspergillus niger* was mineralized in the same period of time.

The results of one experiment showed that the numbers of fungi developing at different temperatures, were highest at 37°C. at first, but later were equalled by those found at 27° and 20°C. According to Jensen (8), however, the numbers are highest at the lower temperatures. This difference can be attributed to the types of fungi present in the different soils. It is significant to record that the Penicillia developing at 37°C. always formed very compact colonies as compared to the corresponding forms developing at 27°C. At low temperatures (6–8°C.) yeasts and Monilia developed in great abundance but apparently were not very active in cellulose decomposition.

The lack of appreciable decomposition of cellulose at 55°C. may be attributed to the absence of anaerobic conditions, which usually favor this process at high temperatures.

SUMMARY

A synthetic medium containing sand and bentonite has been found to be suitable for the study of the various factors affecting the decomposition of cellulose by microörganisms.

Sodium nitrate was a better source of nitrogen than was ammonium sulfate, and a slightly acid initial reaction was most favorable for the decomposition process. Fungi were very prominent initially but were later replaced by bacteria.

A qualitative and quantitative study of the microörganisms developing at different reactions with the two sources of nitrogen indicated that the fungi were more prominent under acid reactions, whereas bacteria were more active under neutral or slightly alkaline reactions. The total number of bacteria after 30 days was much higher in the presence of sodium nitrate than in the presence of ammonium sulfate. The decomposition started more quickly at 37°C., but later the rate of decomposition at 27° and 20° approached that at 37°.

Great differences in fertilizer treatments applied during a period of 25 years exerted no appreciable effect on the microörganisms decomposing cellulose.

REFERENCES

(1) BARTHEL, C., AND BENGTSSON, N. 1924 Action of stable manure in the decomposition of cellulose in tilled soil. *Soil Sci.* 18: 185–200.

(2) BERTRAND, G., AND COMPTON, A. 1911 Influence de la temperature sur l'activité de la cellase. *Bul. Soc. Chim. France* (4) 9: 100.

(3) CHARPENTIER, C. A. G. 1921 Studien über den einfluss des Rindvieh und Pferdestall mistes auf die Zersetzung der Zellulose in der Ackererde. Inaug. Diss. Helsingfors (Quoted from WAKSMAN, S. A. 1927 *Principles of Soil Microbiology*, p. 727. Williams & Wilkins, Baltimore, Md.)

(4) CHOLODNY, N. 1930 Über eine neue Methode zur Untersuchung der Boden Mikroflora. *Arch. Mikrobiol.* 1: 620–652.

(5) CONN, H. J. 1918 The microscopic study of bacteria and fungi in soil. N. Y. Agr. Exp. Sta. Tech. Bul. 64.

(6) DUBOS, R. J. 1928 The decomposition of cellulose by aerobic bacteria. *Jour. Bact.* 15: 223–234.

(7) HEUKELEKIAN, H., AND WAKSMAN, S. A. 1925 Carbon and nitrogen transformations in the decomposition of cellulose by filamentous fungi *Jour. Biol. Chem.* 66: 323–342.

(8) JENSEN, H. L. 1936 Contribution to the microbiology of Australian soils. *Linn. Soc. N. S. Wales Proc.* 61.

(9) KARNICKA, H., AND ZIEMIECKA, J. 1935 Cellulose decomposition in acid soils. *Trans. Third Internatl. Cong. Soil Sci.* 3: 109–111.

(10) MADHOK, M. R. 1937 Synthetic soil as a medium for the study of microbiological processes. *Soil Sci.* In press.

(11) NORMAN, A. G. 1930 The biological decomposition of plant materials. III. Physiological studies of some cellulose decomposing fungi. *Ann. Appl. Biol.* 17: 575–613.

(12) REGE, R. D. 1927 Biochemical decomposition of cellulose. *Ann. Appl. Biol.* 14. 1–43.

(13) ROSSI, G., AND GESUÈ, G. 1930 Di un nuovo indirizzo nello studio biologico del suolo. *Ann. Tech. Agr.* 3: 196–248.

(14) VARTIOVAARA, UNTO 1936 Studies on the metabolism of soil fungi [English summary]. Suomen Maataloustieteel. Seura. Julkaisu. 32.

(15) WAKSMAN, S. A. 1931 Decomposition of the various chemical constituents, etc. of complex plant materials by pure cultures of fungi and bacteria. *Arch. Mikrobiol.* 2: 136–154.

(16) WAKSMAN, S. A., AND HUTCHINGS, I. J. 1937 Associative and antagonistic effects of microörganisms: III. Associative and antagonistic relationships in the decomposition of plant residues. *Soil Sci.* 43: 77–92.

(17) WAKSMAN, S. A., AND SKINNER, C. E. 1926 Microörganisms concerned in the decomposition of cellulose in the soil. *Jour. Bact.* 12: 57–84.

(18) WAKSMAN, S. A., AND STARKEY, R. L. 1924 Influence of organic matter upon the development of fungi, actinomyces and bacteria in the soil. *Soil Sci.* 17: 373–378.

(19) WAKSMAN, S. A., AND STEVENS, K. R. 1930 A system of proximate chemical analysis of plant materials. *Indus. and Engin. Chem. Anal. Ed.* 168–169.

(20) WINOGRADSKY, S. 1924 Sur la microflora autochtone de la terre arable. *Compt. Rend. Acad. Sci.* [Paris]. 178: 1236–1239.

PLATE 1

MICROFLORA OF SOIL TO WHICH CELLULOSE AND NITROGEN WERE ADDED

FIG. 1. After 1 week's incubation, mostly fungi.

FIG. 2. After 3 weeks' incubation, fungal hyphae surrounded by bacteria.

FIG. 3. After 10 weeks' incubation, bacteria prominent.

M. R. MADHOK
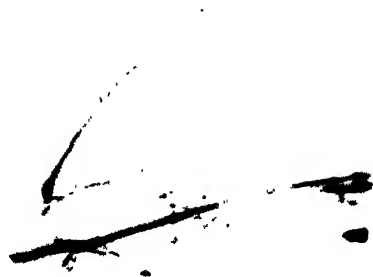
Fig. 1

Fig. 2

Fig. 3

STUDIES IN ELECTRODIALYSIS OF SOILS: II. POLARO-GRAPHIC CURRENT-VOLTAGE CURVES

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It is well known that every reduction takes place at a definite voltage which is characteristic of the respective reducible substance, i.e., its reduction potential. If the voltage of the electrolyzing current is lower than the reduction potential, no reduction takes place, and consequently no current passes through the solution. When the requisite potential is applied, however, the current flowing through the solution is directly proportional to the concentration of the reducible substance. After the pioneer work of Heyrovsky,[1] this principle was applied extensively in the polarographic estimation of metals. The essential parts of the apparatus required for this purpose are a mercury dropping cathode, a 6-volt storage battery connected to a potentiometer for adjusting a uniformly increasing voltage in the electrolytic circuit, and a microammeter for reading the current flowing in the circuit. The current-voltage curves obtained by gradually increasing the voltage and noting the current at every step are characteristic of the nature and concentration of the ions in solution.

It appeared of interest to study the current-voltage curves of soil saturated with different cations and to determine how they compared with those of ordinary soluble salts. Obviously a close analogy between the two sets of curves would indicate a similarity in nature and concentration of the ions.

DESCRIPTION OF THE APPARATUS

Though an automatically operating polarograph is available on the market for such studies, a simpler apparatus assembled from the parts that could be found in the laboratory proved quite satisfactory for our purpose. The apparatus shown in figure 1 consists of a mercury dropping cathode, a potential divider connected to a 6-volt accumulator, and a microammeter.

The results vary slightly, the variation depending on factors such as the size and velocity of mercury drops, the strength of the solution, and the dimensions of the vessel. The same vessel was used throughout this investigation, the

[1] Heyrovsky, J. 1924 Applications of electrolysis by means of the mercury dropping cathode. *Compt. Rend Acad. Sci.* [Paris] 179: 1267.

Heyrovsky, J. 1927 Electrolysis with mercury dropping cathode as an analytical method. *Bul. Soc. Chim. France* 41: 1224.

drop time was adjusted to 3 seconds per drop, and other conditions were kept
constant.   By the help of a side-tube the level of mercury in the vessel was
kept constant.   No precautions were taken to exclude air, as no appreciable
disturbance due to air was noticed.

<center>EXPERIMENTAL</center>

A black cotton soil (P.C. 13 A.T.) of high base-exchange capacity and con-
taining 52 per cent clay was used for these experiments.   All the exchangeable
bases were first removed from the soil by exhaustive treatment with 0.05 N
HCl followed by leaching with water and air drying.   Five-gram portions of
the treated soil were shaken for 48 hours with 50 cc. of alkali solutions of dif-
ferent strengths, solid hydroxides of Ca and Mg being used with 50 cc. of
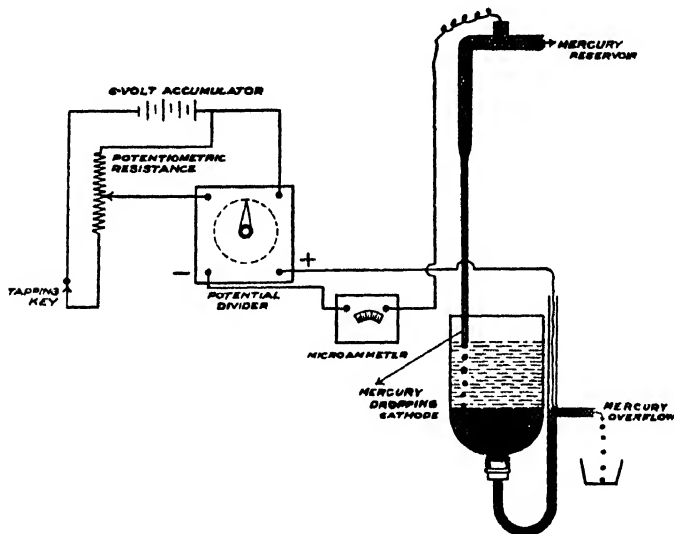
FIG. 1. DIAGRAM OF APPARATUS FOR THE POLAROGRAPHIC EXAMINATION OF SOILS

water.   The duration of shaking was based on a preliminary examination,
which showed that equilibrium between soil and alkali is reached within 48
hours.   The pH values of the soil after treatment with alkalies were deter-
mined by the glass electrode.   For comparison with soluble salts different
concentrations of the chlorides of various metallic ions were used.   The cur-
rent-voltage curves for chlorides of Na, K, Li, NH₄, Ca, Ba, and Mg and for
soils neutralized with corresponding bases are given in figures 2 to 8, which
illustrate in a striking manner the close analogy between the soils and true
salts.

As H-soil is only feebly ionized, very little current passes even at 4 volts.
As it is gradually neutralized, the sharp break at the reduction potential be-
comes more and more apparent.   This observation is in agreement with the

general behavior of weak acids, the salts of which are generally much more ionized than the acid itself. The points in the earlier portions of the curves overlap so much that only two lines corresponding to the lowest and the highest concentrations are given for the sake of clearness. In the first portion of the curves of the chlorides a peculiar kink is noticed which is absent in the
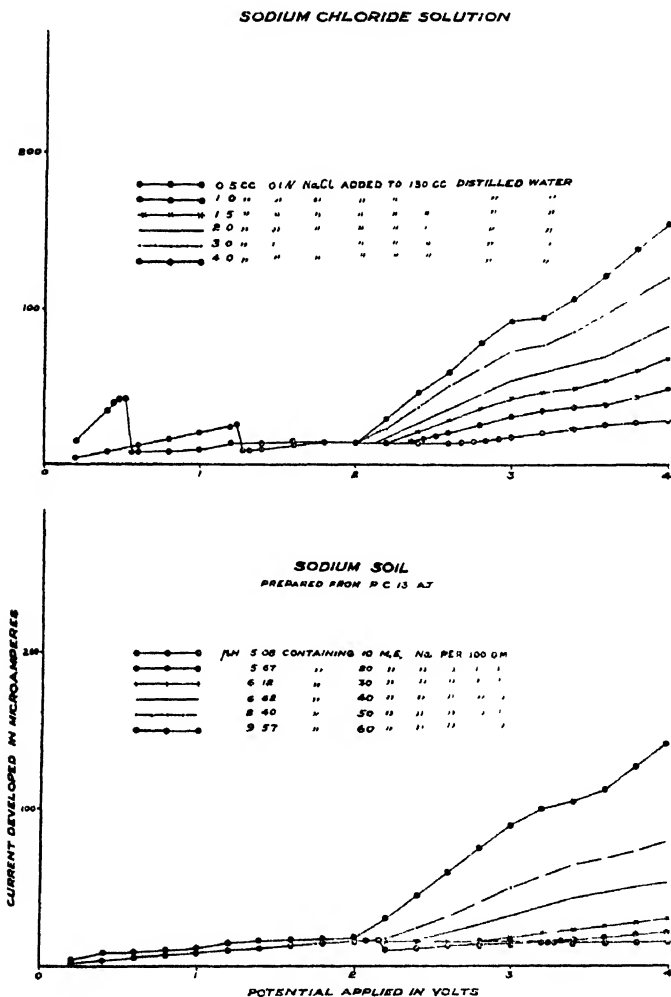
FIG. 2. POLAROGRAPHIC CURRENT-VOLTAGE CURVES FOR NaCl SOLUTION AND FOR Na-SOIL

curves of the soils. No attempt was made to seek an explanation for this phenomenon, which is not very material for the purpose of this investigation.

Another interesting point that emerges from this study is that soils with monovalent cations are more ionized and give steeper current-voltage curves as compared with soils with divalent cations. This is also in general agree-

ment with the behavior of true salts. The data do not admit of quantitative comparison of the degree of ionization of the various cations because, in spite of the precations taken, fluctuations due to temperature changes and to slight alterations in the drop time and in the distance between the electrodes are
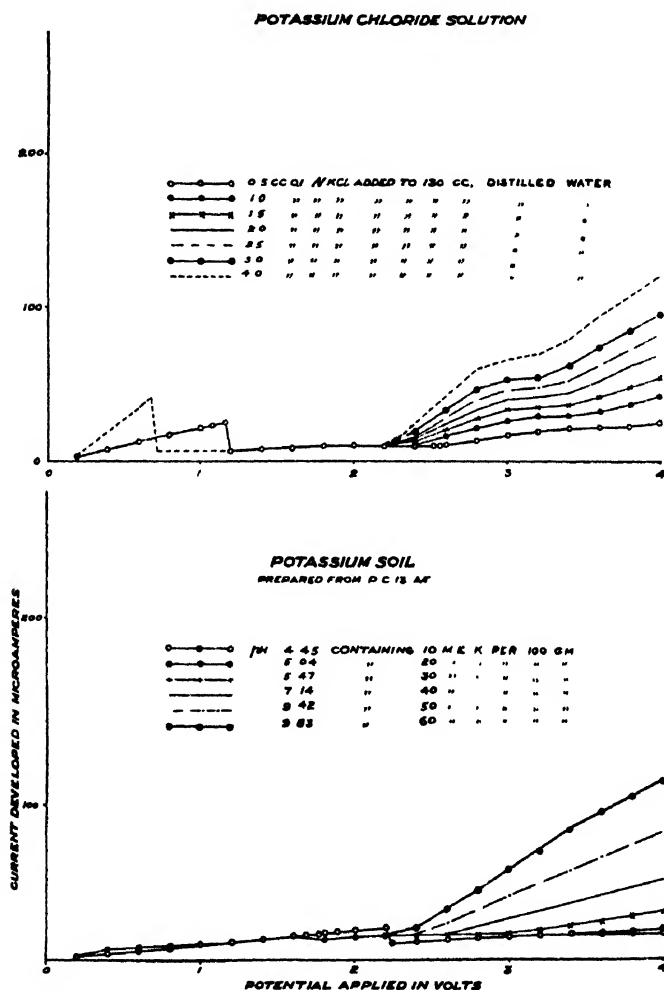
FIG. 3. POLAROGRAPHIC CURRENT-VOLTAGE CURVES FOR KCl SOLUTION AND FOR K-SOIL

bound to occur. The experiments leave no doubt, however, as to the fundamental similarity between true salts and soils saturated with different cations.

In order to determine whether the similarity of polarographic behavior between salts and soil neutralized with different bases, observed in the one soil, was true for other types of soils, two other entirely different types were examined. One was a red soil (P.C. 15 A.T.) from Shillong (Assam), and the

other was an alluvial clay from the Punjab (P.C. 123 A.T.). The results with these soils, given in figure 9, show that the analogy is not restricted to one type of soil but is of general applicability.

The steepness of the second portion of the curve, however, is related to the quantity of exchangeable bases in the soil and not to the pH value; just as in
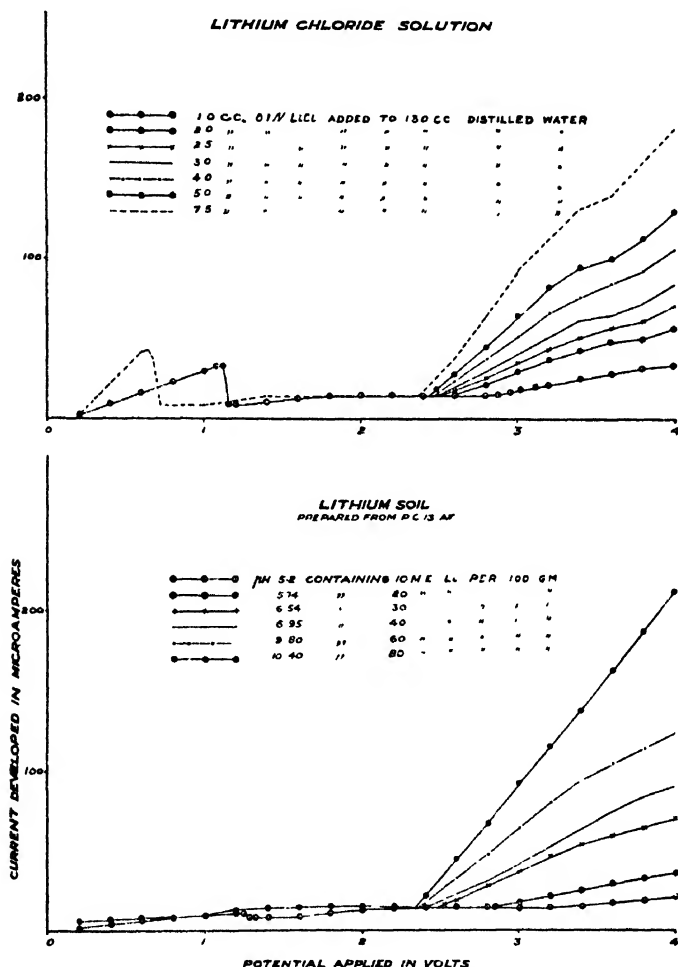
FIG. 4. POLAROGRAPHIC CURRENT-VOLTAGE CURVES FOR LiCl SOLUTION AND FOR Li-SOIL

true salts, it is dependent on the concentration or the quantity of salt in solution. Soil P.C. 15 A.T. requires only a small quantity of alkali for neutralization, and its current-voltage curves show only a slight bend at the reduction potential. Somewhat similar curves are shown by soil P.C. 123 A.T., which requires slightly more alkali than does P.C. 15 A.T. for neutralization.

P.C. 13 A.T. soil was also examined after neutralization partly with Ca(OH)$_2$
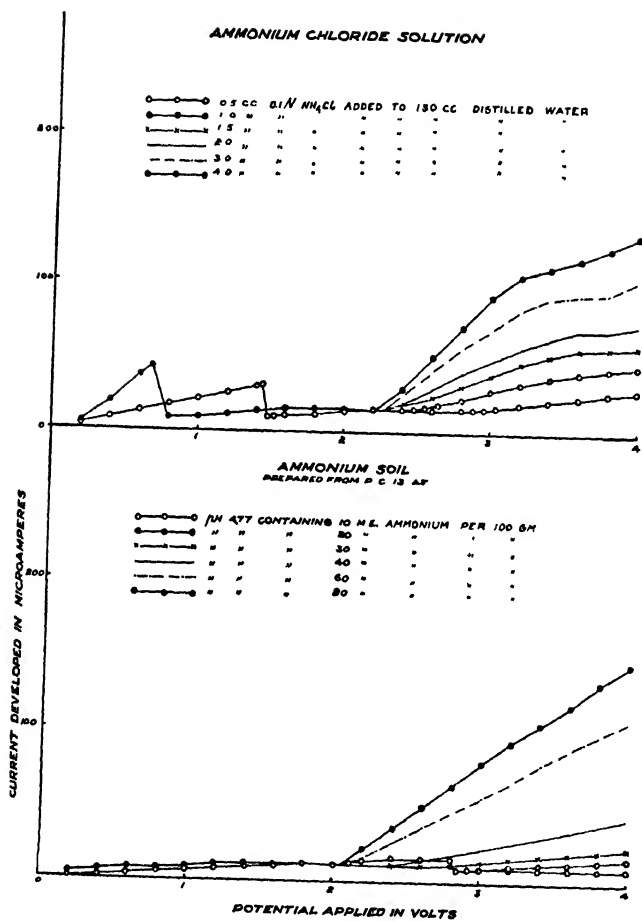
FIG. 5. POLAROGRAPHIC CURRENT-VOLTAGE CURVES FOR NH₄Cl SOLUTION AND FOR NH₄-SOIL
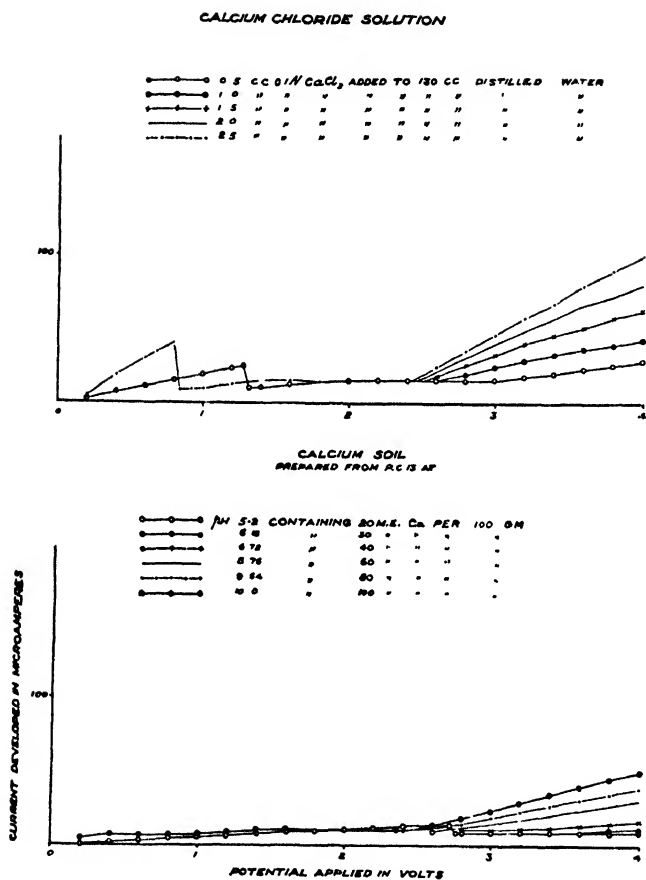
FIG. 6. POLAROGRAPHIC CURRENT-VOLTAGE CURVES FOR CaCl₂ SOLUTION AND FOR Ca-SOIL

BARIUM CHLORIDE SOLUTION

BARIUM SOIL
PREPARED FROM P C 13 AT

FIG. 7. POLAROGRAPHIC CURRENT-VOLTAGE CURVES FOR BaCl₂ SOLUTION AND FOR Ba-SOIL
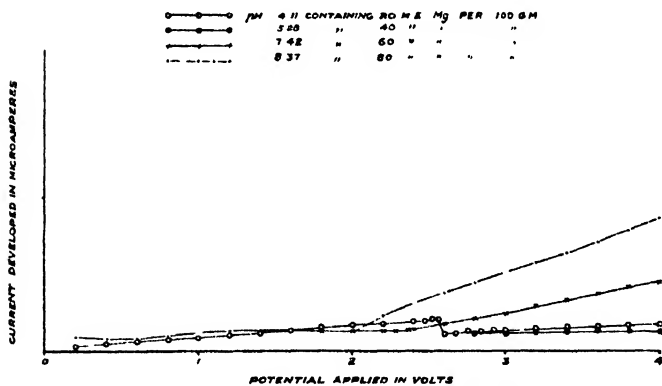
FIG. 8. POLAROGRAPHIC CURRENT-VOLTAGE CURVES FOR MgCl₂ SOLUTION AND FOR Mg-SOIL

and partly with NaOH. It was thought that the polarographic measurements might show well-defined bends at the Na and Ca reduction potentials. Figure 10 shows no such breaks. The original P.C. 13 soil without the acid treatment was also examined. The curve obtained with this soil sample resembled that of Ca-soil. This is in agreement with the observation that calcium forms by far the largest percentage of the exchangeable bases in this soil.

It is not possible at present to determine the quantity of exchangeable bases in a given soil from polarographic analysis. Perhaps this might be possible
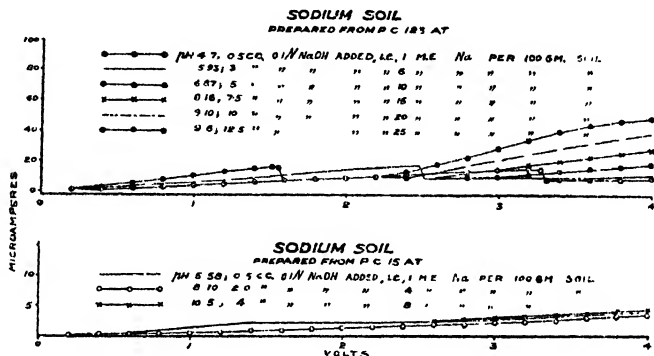
FIG 9. POLAROGRAPHIC CURRENT-VOLTAGE CURVES FOR A RED SOIL AND AN ALLUVIAL CLAY NEUTRALIZED WITH Na

(Compare with the curve for the Na-soil shown in figure 2)
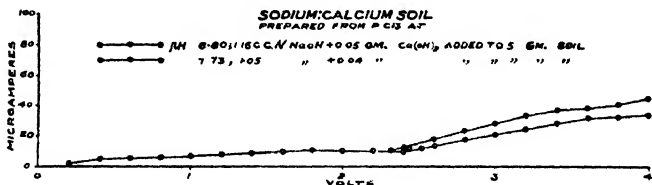
FIG. 10. POLAROGRAPHIC CURRENT-VOLTAGE CURVES FOR A SOIL NEUTRALIZED PARTLY WITH Ca(OH)₂ AND PARTLY WITH NaOH

with a more refined technic and with the help of automatic recording instruments.

SUMMARY

Polarographic current-voltage curves for soils having different exchangeable bases bear a close resemblance to such curves for soluble salts of corresponding bases. It appears, therefore, that exchangeable bases in soils exist in chemical combination and not in the absorbed state.

ERRATUM

Synthetic soil as a medium for the study of certain microbiological processes, by M. R. Madhok

P 321   Table 2, heading column 4, $NO_2 - N$ should read $NO_3 - N$;
heading column 7, $NO_3 - N$ should read $NO_2 - N$.

STUDIES ON THE ROOT-NODULE BACTERIA OF WILD LEGU-
MINOUS PLANTS IN WISCONSIN[1]

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University of Wisconsin

A recent survey of the wild leguminous plants of Wisconsin has offered un-
usual opportunity for the isolation and study of the root-nodule bacteria of
these plants. The cultural and morphological characteristics of these or-
ganisms and their cross-inoculation group relationships have been investigated,
and it is the results of these studies which are presented in this paper.

Since the publication in 1932 of Fred, Baldwin, and McCoy's monograph
(6), the contributions of Carroll (3), Conklin (4), Raju (15), and Allen and Allen
(1) have extended considerably the literature upon the cross-inoculation group-
ing of root-nodule bacteria from plants growing in widely separated parts of
the world. They have added 140 new species to the 109 plants placed in the
several cross-inoculation groups by workers before 1932, and Carroll and Raju
have also added to the number of cross-inoculation groups themselves.

The floras of the geographic regions involved in these recent studies differ
so widely among themselves and from the leguminous flora of Wisconsin that
there has been little duplication in the plants classified. The few similarities
and conflicts noted between the results obtained by earlier investigators and
the data presented in this paper will be discussed in more detail in another
section of this paper.

METHODS

Collection of nodules

Root nodules from different species of leguminous plants growing naturally
in Wisconsin[3] were collected, and the plants from which these nodules were

[3] A plant "wild" in Wisconsin is not necessarily one native to Wisconsin; indeed, many
of the leguminous species now found growing wild have been introduced or have migrated
into different parts of this state, where they have established themselves.

obtained were identified. Isolation of the organisms from the nodules was begun as soon as it was possible to bring the nodules to the laboratory from the field.

Roots of the non-nodulated leguminous plants *Cassia marilandica,Gleditsia triacanthos*, and *Gymnocladus dioica* and roots of the "nodulated" non-leguminous plants *Alnus mollis* and *Myrica caroliniana* were also collected and sent to the laboratory.

Isolation of the root-nodule bacteria

The nodules were first washed free of soil. Those that were desiccated were allowed to soak in sterile distilled water for 3 to 5 hours. They were then treated with 95 per cent alcohol for 2 minutes, after which their surfaces were disinfected by immersing the whole nodules in a 1:1000 aqueous solution of mercuric chloride. Large nodules, or nodules with many lobes or roughened surfaces, were exposed to the mercuric chloride solution for 5 minutes, and small nodules, for only 3 minutes. After several successive washings in sterile distilled water, each of the treated nodules was transferred to a sterile Petri dish to which about 1 cc. of sterile distilled water had been added. The nodule was then crushed and well macerated in this water, from which serial loop dilutions were made into a second Petri dish, and from that into a third, containing sterile distilled water. Yeast water-mannitol-mineral salts agar containing congo red indicator was used as the plating medium (7). When the number of nodules on each specimen collected was large enough, the contents of at least three of the nodules were plated out, separately, in this manner.

The "nodules" of Alnus and of Myrica and portions of the rootlets of the non-nodulated leguminous plants which appeared to be swollen or thickened were subjected to the same treatment in attempts to isolate rhizobia from them.

Plates were incubated at room temperature for 7 to 14 days. Well-isolated colonies characteristic of rhizobia were then transferred to freshly-prepared slants of yeast water-mannitol-mineral salts agar containing calcium carbonate.

Cultural and morphological characteristics of the rhizobia

The purity and the cultural characteristics of the isolations were determined by cultivating the isolations in litmus milk, in veal infusion broth, in Clark and Lub's liquid medium, on calcium glycero-phosphate agar, and on yeast water-mannitol-mineral salts agar.[4] In all tests, stock cultures of each of the

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[4] The formula for preparing litmus milk is given by Fred and Waksman (7); the method for preparing veal infusion broth, by the Society of American Bacteriologists, (18); for Clark and Lub's liquid medium, by the American Public Health Association, (2); and that for calcium-glycero-phosphate agar, by Fred, Baldwin, and McCoy (6) adapted from Müller and Stapp (14).

now-recognized Rhizobium species were employed as standards of comparison for the unknown cultures.

The morphological characteristics of the organisms were studied in order to check the purity and to aid in the identification of the different cultures. Observations were made on the Gram character, the relative size and shape, and the nature of the flagellation of the organisms. Gram stains were made, the Kopeloff and Beerman (11) modification being used, with yeast water-mannitol-mineral salts agar slant cultures 2, 5, and 12 days old. The sizes of the cells were determined from these Gram-stained smears with the aid of a filar micrometer.

The technique developed by Gray (9) was used in making the flagella stains. The organisms were grown for 2 days at a time in two successive transfers on Laird's (13) modification of Hitchner's medium. The slow-growing types of organisms were then transferred to Koser's (12) liquid sodium citrate medium, and the fast-growing types were transferred to liquid sodium succinate medium. The organisms were grown for 2 more days in these media. Excellent flagella mounts, almost free of debris, were obtained by this method of cultivation.

Cross-inoculation tests

The cultures were tested for their ability to form nodules on species of leguminous plants belonging to the major cross-inoculation groups, and, when possible, on seedlings of the same species of plants as those from which they had been isolated. In some instances where seeds of the original hosts could not be obtained or could not be made to germinate, seeds of other species in the same genus were used.

These greenhouse tests were attempted only during those periods of the year when growing conditions were conducive to best development of the plants, that is, during spring, summer, and early fall. Each culture was tested, in triplicate, at least three times agains its homologous host, or a closely related species, for its ability to form nodules, and at least three times more against seedlings of plant species commonly used as representatives of the major cross-inoculation groups[6] to which earlier tests indicated the cultures might belong. Further, cultures obtained from plant species eventually shown to belong to some of the minor cross-inoculation groups, and from the several unclassifiable "unknowns," were tested against seedlings of plants from all the major and from several of the minor cross-inoculation groups. The following species were employed in these tests:

[6] For convenience in discussing them, the cross-inoculation groups have been divided arbitrarily into the "major" and the "minor" cross-inoculation groups. The "major" groups include those groups the organisms specific for which have been given definite scientific names—the alfalfa, clover, pea, bean, soybean, and lupine groups, and, in concession to its size, the cowpea group. The "minor" groups, on the other hand, include all those other less well-known groups which enjoy the anonymity of Roman numerals in the classifications that have appeared in the literature so far.

GROUP	SPECIES	GROUP	SPECIES
Alfalfa-sweet clover	*Medicago sativa* (Grimm)	Cowpea	*Vigna sinensis*
			Lespedeza sericea
			Lespedeza stipulaceae
True clover	*Trifolium pratense* (Common red)		*Lespedeza striata*
			Lespedeza virginica
			Baptisia leucantha
Pea and vetch	*Pisum sativum* (Alaska)		*Desmodium canadense*
			Desmodium illinoense
	Lathyrus japonicus		*Desmodium purpureum*
	Lathyrus odoratus		*Desmodium tortuosum*
	Vicia sativa		
	Vicia villosa	Lupine	*Lupinus perennis*
	Vicia Faba		*Lupinus Hartwegii*
Bean	*Phaseolus vulgaris* (Refugee stringless)	Locust	*Robinia Pseudo-Acacia*
		Amorpha	*Amorpha fruticosa*
Soybean	*Glycine hispida* (Manchu)		*Amorpha herbacea*
Dalea	*Dalea alopecuroides*	Strophostyles	*Strophostyles helvola*

Seeds of the plants being tested were treated with 95 per cent alcohol for 2 minutes, and then with a 1:1000 aqueous solution of mercuric chloride for 3 to 5 minutes, after which they were washed six times with sterile distilled water. This treatment succeeded in providing seed free of rhizobia.

All plants were grown in sterilized, nitrogen-poor, glacial pit sand, held in half-gallon glazed earthenware pots. At the time of planting, the seeds were inoculated with water suspensions of the organisms freshly prepared from duplicate 14-day-old agar slant cultures. About 25 cc. of the suspension was poured over the seeds in each of the three pots in the set devoted to the culture being studied.

About one-fifth as many pots as were provided for the cultures were retained as uninoculated controls and were spaced at regular intervals among the inoculated sets. The plants received sterile Crone's nitrogen-free solution [Bryan's modification (7)] every 2 weeks and sterile distilled water whenever it was needed.

Two or three species of plants, usually from the same cross-inoculation group and of the same habit of growth, were grown together in many pots. Thus, in studies on the pea and vetch group, seedlings of *Pisum sativum*, *Vicia sativa*, *Vicia Faba*, and *Lathyrus japonicus* were grown together in combinations of two or three species. Many soybean and cowpea plants were grown together to show the occurrence of crossing-over among strains of rhizobia obtained from plants in the cowpea group. Small slow-growing plants, like Lespedeza, Amorpha, and Astragalus species, were not grown in the same pots with bushy fast-growing plants like soybeans and cowpeas but were grown separately, either alone or in combination with other plants having the same habits of growth.

When the plants were from 4 to 6 weeks old they were washed free of sand and examined for the presence or absence of nodules. If the uninoculated control plants were not nodulated it was assumed that the formation of nodules on plants inoculated with a certain culture was directly ascribable to that culture and that the bacteria and the leguminous plants they had nodulated belonged to the same cross-inoculation group. Similarly, if under the same conditions certain cultures did not form nodules it was assumed that the bacteria and the plants belonged to different cross-inoculation groups. These conclusions were drawn from observations made upon at least 12 plants of each species in each set of three pots devoted to the culture being tested.

<div style="text-align:center">

RESULTS

Morphological and cultural characteristics

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The morphological and cultural characteristics of strains from each of the genera included in these studies are summarized in table 1. The cultures isolated from the species of plants making up each genus shared these characteristics to such a degree that their general similarity permits of grouping them by genera rather than by individual species. The responses of the different strains to inoculation into veal infusion broth and into Clark and Lub's liquid medium are not presented in the table, as these media were used primarily in purity tests to show the possible presence of contaminants in the isolations: the rhizobia either do not grow at all or grow with only a uniform clouding of the media, whereas contaminating types, among which *Achromobacter radiobacter* is most common, grow profusely throughout the media and form heavy pellicles as well. Litmus milk, calcium glycero-phosphate agar, and yeast water-mannitol-mineral salts agar, although also capable in some degree of indicating contamination, offer more valuable and consistent characters for the differentiation of species.

Morphologically the root-nodule bacteria that were studied showed considerable variation. They were all Gram-negative, uniformly-stained rods when young, ranging in length from the very small (1.0–1.5 μ), almost coccoid types found in cultures obtained from Amorpha, Astragalus, Glycyrrhiza, Oxytropis, and Psoralea plants, to the relatively long, slender rods (measuring from 2.5–3.5 μ) observed in cultures from Lathyrus, Vicia, Medicago, and Trifolium species. Organisms isolated from Amphicarpa, Apios, Baptisia, Cassia, Crotalaria, Desmodium, and Lespedeza species, later shown to belong to the cowpea group, and from Lupinus, Robinia, and Strophostyles species were medium-long types, ranging from 1.5 to 2.5 μ in length. These measurements were made on organisms from the 2-day cultures.

Gram stains of the organisms from 2-, 5-, and 12-day-old cultures showed a progressive vacuolation, granulation, and disintegration of the cells. In almost all instances a few uniformly stained rods were still present in the 12-day-old cultures, although most of them had given way to vacuolated banded rods and numerous small coccoid forms. This variety of form is in agreement

TABLE 1

Morphological and cultural characteristics of strains from wild leguminous plants (grouped by genera)

GENUS	NUMBER OF STRAINS	MORPHOLOGICAL CHARACTERISTICS		CULTURAL CHARACTERISTICS		
		Size and shape	Flagellation	Litmus milk (6 weeks)	Calcium glycero-phosphate (4 weeks)	Yeast water-mannitol-mineral salts agar (4 weeks)
Medicago	2	Long rods	Peritrichous	Very acid, with zone	Moderate, watery, opaque	Abundant, gummy, opaque
Trifolium	2	Long rods	Peritrichous	Very alkaline, with zone	Scant, dry, opaque	Abundant, gummy, opaque
Lathyrus	20	Medium to long rods	Peritrichous	Very alkaline, with zone	Scant, dry, opaque	Abundant, gummy, opaque
Vicia	11	Long rods	Peritrichous	Very alkaline, with zone	Scant, dry, opaque	Abundant, gummy, opaque
Amphicarpa	5	Medium to long rods	Monotrichous	Very alkaline, no zone	Scant, watery, transparent	Moderate, watery, transparent
Apios	2	Short to medium rods	Monotrichous	Very alkaline, no zone	Scant, dry, opaque	Moderate, gummy, opaque
Baptisia	6	Short rods	Monotrichous	Very alkaline, no zone	Scant, dry, opaque	Scant, moist, opaque
Cassia	1	Medium rods	Monotrichous	Very alkaline, no zone	Scant, watery, transparent	Moderate, gummy, opaque
Crotalaria	2	Medium rods	Monotrichous	Very alkaline, no zone	Moderate, watery, opaque	Moderate, gummy, opaque
Desmodium	15	Short to medium rods	Monotrichous	Very alkaline, no zone	Scant, moist, opaque	Scant to moderate, watery to gummy, transparent to opaque
Lespedeza	7	Short to medium rods	Monotrichous	Very alkaline, no zone	Moderate, watery, opaque	Scant, watery, transparent
Lupinus	4	Medium rods	Monotrichous	Very alkaline, no zone	Scant, moist, opaque	Moderate, gummy, opaque
Amorpha	5	Very short rods	Monotrichous	Very alkaline, with zone	Moderate, moist, opaque	Abundant, gummy, opaque
Petalostemum	6	Short to medium rods	Monotrichous	Very alkaline, with zone	Abundant, gummy, opaque	Abundant, gummy, opaque
Astragalus	5	Very short rods	Monotrichous	Very alkaline, with zone	Moderate, moist, opaque	Abundant, gummy, opaque
Glycyrrhiza	1	Very short rods	Monotrichous	Very alkaline, with zone	Scant, dry, opaque	Scant, watery, transparent
Oxytropis	2	Very short rods	Monotrichous	Very alkaline, with zone	Scant, moist, transparent	Scant, watery, transparent
Psoralea	1	Very short rods	Monotrichous	Acid, no zone	Scant, dry, transparent	Scant, watery, transparent
Robinia	4	Short rods	Monotrichous	Acid to alkaline, with zone	Abundant, gummy, opaque	Abundant, gummy, opaque
Strophostyles	7	Short rods	Monotrichous	Very alkaline, with zone	Abundant, gummy, opaque	Abundant, gummy, opaque
Tephrosia	4	Short to medium rods	Monotrichous	Very alkaline.		

with the claims of earlier workers that the rhizobia exhibit a pleomorphism akin to life-cycle changes.

Flagella stains of the root-nodule bacteria isolated from Medicago, Trifolium, Lathyrus, and Vicia species showed that the flagellation of these organisms is peritrichous. The short to medium rods from the genera included in the cowpea group, and from the one species of lupine, possess only one flagellum each, usually extending from one "corner" of the cell. The organisms from the plants in the nine other genera—Amorpha, Petalostemum, Astragalus, Glycyrrhiza, Oxytropis, Psoralea, Robinia, Strophostyles, and Tephrosia—are monotrichous.

The cultural characteristics of the organisms show less marked variation. Only the strains obtained from *Medicago lupulina* and *Psoralea esculenta* produced a lasting acid reaction in milk; cultures from Robinia produced only an incipient acidity, which was soon superseded by a definite alkalinity. Strains from all the other genera, regardless of their cross-inoculation affinities, produced strongly alkaline reactions in litmus milk.

The strains isolated from Lathyrus and Vicia species were characteristic of *Rhizobium leguminosarum* cultures: they produced a marked serum zone and strong alkalinity in litmus milk, grew poorly on calcium glycero-phosphate agar, but throve on yeast water-mannitol-mineral salts agar, upon which their growth was profuse, gummy, and opaque.

Cultures from genera commonly classified in the cowpea group—Baptisia, Cassia, Crotalaria, Desmodium, and Lespedeza—and from two new genera recently added to the cowpea group—Amphicarpa and Apios—elicited a strongly alkaline reaction in litmus milk but formed no serum zones, a feature characteristic of the Rhizobium for cowpea. Their similarity to rhizobia isolated from cowpea plants was still more strongly brought out by the general scantiness of their growth on both calcium glycero-phosphate and yeast water-mannitol-mineral salts agar.[6] The cultures from Lupinus gave much the same responses.

The organisms from the remaining genera, as yet unaffiliated with any of the cross-inoculation groups, present suggestive resemblances to representatives of these groups, although their interrelationships apparently go no further than that. Cultures from Glycyrrhiza and Oxytropis, although producing a strongly alkaline reaction and a definite serum zone in litmus milk, grow poorly on calcium glycero-phosphate and yeast water-mannitol-mineral salts agar; this degree of growth upon the two agar media is the only cultural feature

[6] It was found, as a result of the studies of Tatum, Peterson, and Fred (19) and of Sarles and Reid (17), that the addition of potato extract to yeast water-mannitol-mineral salts agar (with calcium carbonate omitted) enabled the organisms from the soybean, cowpea, and lupine groups to grow much more profusely and to produce more gum than they were able to in the standard medium. This modified medium is, accordingly, the one upon which these organisms are now cultivated. Characteristics presented in the table, however, refer to the type of growth obtained in the standard medium

brought out in these studies in which they differ significantly from organisms isolated from Amorpha, Astragalus, Petalostemum, Robinia, Strophostyles, and Tephrosia plants. The organisms obtained from Astragalus species are similar, too, in these cultural reactions, to those cultures of rhizobia isolated from *Astragalus sinicus* by the Japanese workers Itano and Matsuura (10).

Organisms isolated from the swollen rootlets of the non-nodulated leguminous species *Cassia marilandica, Gleditsia triacanthos*, and *Gymnocladus dioica* and from the "nodulated" non-leguminous plants *Alnus mollis* and *Myrica caroliniana* were found, in the course of the cultural tests, not to be rhizobia, and they were eventually discarded.

Cross-inoculation tests

The cultures which, to all appearances, reacted in these cultural tests as did typical Rhizobium cultures, represented isolations from nodules of 44 species of leguminous plants. Of these, at the time they were obtained, 14 had already been studied by earlier workers, leaving 30 new species to be classified into cross-inoculation groups.[7]

One of the cultures from *Cassia marilandica*, in spite of its atypical reactions in the cultural tests, was employed in several greenhouse experiments on soybean, cowpea, lespedeza, and *Cassia fasciculata* seedlings, but failed to produce nodules on any of these plants.

Table 2 presents the distribution of certain of these wild plants among the cross-inoculation groups. Table 3 lists those species which, for one reason or another, could not be found to cross with representatives of any of the other cross-inoculation groups that were tried. For the plant names the nomenclature employed by Gray (8) has been followed, except where recent changes in the nomenclature of species have been made, in which cases the older names have been enclosed in parentheses.

DATA AND COMMENT

Fifteen of the wild leguminous species found in Wisconsin are placed in the cowpea group. They are members of seven genera, five of which have long been represented in the cowpea group by some of the species coming within the scope of this paper and by other better known species. Two other genera have been added recently to the list of those plants the root-nodule organisms of which will form nodules on *Vigna sinensis*; in these instances, then, our studies have confirmed Carroll's addition of *Apios americana* (*A. tuberosa*), and Conklin's addition of *Amphicarpa bracteata* and *Crotalaria sagittalis* to the cowpea group, while at the same time adding a new variety of the Amphicarpa species, *A. bracteata* var. *Pitcheri*, to the group.[8] These results in-

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[7] Since these studies were begun Carroll (3) has placed one of these new species, *Apios americana*, in the cowpea group, and Conklin (4) has added *Amphicarpa bracteata* and *Crotalaria sagittalis* to the cowpea group and *Lathyrus japonicus* var. *pellitus* to the pea and vetch group

[8] Cross-inoculation tests with rhizobia isolated from *Baptisia alba* (L.) R. Br. collected in Indiana indicate that this species, too, can be added to the cowpea group.

TABLE 2

Distribution of wild leguminous plants among the known cross-inoculation groups

Cowpea Group

*‡*Amphicarpa bracteata* (L.) Fernald (*A. monoica*)
Amphicarpa bracteata var. Pitcheri (T. and G.) Fassett (*A. Pitcheri*)
†*Apios americana* Med. (*Apios tuberosa*)
Baptisia leucophaea Nutt.
Baptisia leucantha T. and G.
Cassia fasciculata Michx.
‡*Crotalaria sagittalis* L.
Desmodium accuminatum (Michx.) DC.
Desmodium bracteosum var. longifolium (Michx.) DC.
Desmodium canadense (L.) DC.
**Desmodium canescens* (L.) DC.
Desmodium Dillenii Darl.
**Desmodium illinoense* Gray
Lespedeza capitata Michx.
Lespedeza violacea (L.) Pers.
**Lespedeza virginica* (L.) Britton

Pea and Vetch Group

Lathyrus japonicus Willd. *var. glaber* (Ser) Fernald⎱
‡*Lathyrus japonicus* Willd. *var. pellitus* Fernald ⎰ (*L. maritimus*)
Lathyrus ochroleucus Hook
Lathyrus palustris var linearifolius Ser.
Lathyrus palustris var myrtifolius (Muhl) Gray
*Lathyrus venosus Muhl.
Vicia americana Muhl
**Vicia angustifolia* (L) Reichard.
Vicia caroliniana Walt.
Vicia tenuifolia Roth
*Vicia villosa Roth.

Locust Group

**Robinia Pesudo-Acacia* L.
Robinia viscosa Vent.

Amorpha Group

**Amorpha canescens* Pursh.
**Amorpha fruticosa* L var. *angustifolia* Pursh.

Lupine Group

*Lupinus perennis L. *var occidentalis* Wats.

Strophostyles Group

**Strophostyles helvola* (L.) Britton
Strophostyles leiosperma Piper (*S. pauciflora*)

Alfalfa-Sweet Clover Group

**Medicago lupulina* L.

True Clover Group

*Trifolium repens L.

* Species studied by early workers and listed by Fred, Baldwin, and McCoy (6).
† Species studied by Carroll (3).
‡ Species studied by Conklin (4).

dicate that a great number of the wild leguminous plants growing in this region belong to the cowpea group.

Whereas in the leguminous flora of the southern United States, insofar as it has been reported, the pea and vetch group apparently has but little representation, it has a far greater membership in the more northern plant life as it is encountered in Wisconsin.   In this region members of the genera Lathyrus and Vicia were as frequently observed and collected as were members of any of the other genera.   Nine species, two of them having two varieties each, are placed in the pea and vetch group by these studies.   Five of these species and one variety of a sixth are classified in the pea and vetch group for the first time, and Conklin's addition of *Lathyrus japonicus* var. *pellitus* is confirmed.[9]

Two new species appear to be representatives of as many minor cross-inoculation groups: *Robinia viscosa* is placed with *R. Pseudo-Acacia* in the Locust group, and *Strophostyles leiosperma* is placed with *S. helvola* in the small group which bears their generic name.

TABLE 3

Plants at present unassigned to any cross-inoculation group

Astragalus alpinus L.
Astragalus canadensis L.
Astragalus caryocarpus Ker.
Astragalus neglectus (T. and G.) Sheldon
Glycyrrhiza lepidota Pursh.
Oxytropis chartacea Fassett.
Petalostemum candidum Michx
Petalostemum purpureum (Vent.) Rydb.
Petalostemum villosum Nutt.
Psoralea esculenta Pursh.
Tephrosia virginiana (L.) Pers. *var. holosericea* (Nutt) T and G

Organisms isolated from *Amorpha canescens* and *Amorpha fruticosa* formed nodules on Amorpha plants only and would not cross with representatives of any of the other cross-inoculation groups.   The status of the minor Amorpha group, therefore, remains unaffected by these studies.

Eleven species, belonging to six genera, are as yet unassociated with any particular bacterial-plant groups and require further study before their cross-inoculation relationships can be established definitely.   Both the plants and the bacteria obtained from these plants exhibit a number of peculiarities which make them difficult to study and to classify.

The very rarity of some of these species in Wisconsin offers an impediment to their classification.   They are found in such small numbers and in such isolated ranges that it is as much a problem to obtain seeds from them as it is to

[9] *Vicia micrantha* Nutt., collected in Arkansas may also be added to the pea and vetch group on the basis of evidence gathered from greenhouse tests with rhizobia isolated from its root nodules.

find nodules on their roots.   This was found to be true especially of *Oxytropis chartacea* and of *Psoralea esculenta*.   *Oxytropis chartacea* is, indeed, a newly-discovered species (5), an epibiotic endemic to only two localities in northern Wisconsin.   Its nearest relatives, other species of Oxytropis, are found in the Rocky Mountains and in the region, famous for its endemics, about the Gulf of St. Lawrence, hundreds of miles removed from its Wisconsin habitats.   Yet this isolated species was found to possess nodules, bacteria from which are unable to form nodules on any of the species of *Leguminosae* tested.   The one species of Psoralea was collected in only two localities and was not found with seeds; consequently the nodule-forming power of the culture from Psoralea could not be established.

The species of Petalostemum rarely set seed in this country.   Here again it was impossible to test the ability of cultures isolated from the roots of Petalostemum species to form nodules on their specific hosts because bacteria-free young plants could not be grown for these tests.   *For this reason, too,* cultures isolated from *Amorpha canescens* had to be tested against seedlings of *A. fruticosa*, which were fortunately available in sufficient numbers.

<div align="center">DISCUSSION</div>

It is evident, then, that the classification of these eleven species as "unknowns," at least temporarily, is attributable as much to their inability to produce viable seed as it is to the inability of their bacterial symbionts to form nodules on the roots of plants in the other cross-inoculation groups.   It may be, of course, that some of these species belong to their own restricted and self-sufficient groups and that others may yet be shown to belong to those few minor cross-inoculation groups which were not included in these tests.

It is quite possible, for instance, that the Petalostemum species may belong in the same group as do the two Amorpha species: members of the two genera grow in identical habitats, often in the absence of any other species of leguminous plants; they possess the same types of root systems, and produce nodules identical in appearance, structure, and position on the root-systems of the different hosts.   Culturally, physiologically, and morphologically the organisms from the plants in the two genera bear marked resemblances.   Cultures isolated fom Petalostemum nodules have also, in two tests, formed nodules on *Amorpha fruticosa* seedlings.   But the uncertainty of the specificity of the Petalostemum cultures and the prohibition placed upon the reciprocal crossing of Amorpha cultures with Petalostemum plants by the lack of these plants make such a joint grouping inadvisable at this time.

The behavior of the cultures isolated from Astragalus plants is also unique. Culturally and physiologicaly the organisms resemble very much cultures of *Rhizobium leguminosarum* or *Rhizobium trifolii*, although morphologically their small size keeps them distinct from these two species.   The long, slender, finger-like, white nodules they form on Astragalus plants, however, are very suggestive of nodules formed on clover species by *Rhizobium trifolii*.   Never-

theless, the cultures from Astragalus plants did not form nodules on any of the other plants that were tested, nor would cultures from plants in other cross-inoculation groups form nodules on Astragalus plants. These results, although they do not warrant the establishment of a new cross-inoculation group for Astragalus species, do lend weight to the probability that Astragalus species make up their own select cross-inoculation group.

Although Allen and Allen (1) have placed three tropical Tephrosia species (*Tephrosia candida, T. purpurea, T. noctiflora*), and thereby a new genus, in the cowpea group, cultures isolated from *Tephrosia virginiana* var. *holosericea*, found in Wisconsin, did not form nodules on the cowpea plant or on any other plant tested. It is not likely that here is another instance of a genus being divided between two cross-inoculation groups, as is the case with the genus Phaseolus, and the genus Vicia as it is separated by Carroll; further greenhouse tests upon the cultures from the Wisconsin species of Tephrosia are being made in order to clarify their position.

The literature on the status of the cowpea group has been discussed in some detail by Fred, Baldwin, and McCoy (6). Additions and annotations have been made to this review in the more recent papers of Carroll (3), Walker and Brown (20), Allen and Allen (1), Raju (15), and Reid (16).

Carroll and Walker and Brown are of the opinion that, because of the frequent interchangeability of soybean and cowpea strains of rhizobia on soybean and cowpea plants, the two groups should be united, *Rhizobium japonicum* being recognized as the specific agent of infection. Resemblances in the physiological and morphological characteristics of organisms from both hosts lend further strength to this contention.

Allen and Allen, on the other hand, in their extensive tests with tropical *Leguminosae* in the cowpea group, on no occasion observed nodules produced on soybean plants inoculated with strains isolated from these tropical plants, even though the root systems of cowpea plants, intertwined with those of the soybean plants, were well nodulated. These observations, made over a period of 5 years under the very equable meteorological conditions afforded by the Hawaiian climate, led them to be skeptical of any move to consolidate the soybean and cowpea groups.

In these studies on the rhizobia from the wild leguminous plants of Wisconsin, and in Reid's investigations upon the infective ability of rhizobia from the soybean, cowpea, and lupine cross-inoculation groups, many soybean and cowpea plants have been grown together in the same containers, both with and without other species of plants. Many of their root systems showed an irregularity of nodulation that could be ascribed both to the season of the year and to the source of the culture used as an inoculum. In the fall or winter, when growth of the plants was very poor, there was little crossing over, nodulation being confined chiefly to the homologous host or to plants closely related to it; when growing conditions were favorable, as in summer, crossing among soybean, cowpea, and lupine plants and strains was widespread and

generally inconsiderate of narrow specificities (16). These investigations carry a few steps farther the observations made by Raju (15), who noted that the members of the cowpea group are "closely related to the soybean and the lupine (as well as to dalea and dhaincha) as far as nodule production is concerned."

Some strains isolated from wild plants, however, showed distinct preferences for one host or the other, even when the plants were grown together in the same pots. Thus, strains from Baptisia plants did not form nodules on soybean plants but readily induced nodulation on cowpea and lupine plants. On the other hand, Desmodium strains more frequently infected soybean plants than they did cowpea plants and never formed nodules on lupines. Similarly, Lespedeza and Lupinus strains preferred their own hosts first; cowpea plants were almost as frequently infected; and soybean plants were infected by these strains to the least degree.

TABLE 4

Preference of strains isolated from wild leguminosae for the type-plants of the soybean, cowpea, and lupine groups

SOURCE OF BACTERIA	SOYBEAN (*Glycine hispida*)	COWPEA (*Vigna sinensis*)	LUPINE (*Lupinus hartwegii*)
Amphicarpa .	+++	++++	−
Apios.	++++	++++	−
Baptisia	−	++++	+++
Cassia . .	+	++++	−
Crotalaria . . .	+++	++++	++
Desmodium .	+++	++	−
Lespedeza	++	+++	+++
Lupinus .	+	+++	++++

++++ indicates that in 75–100 per cent of the tests nodules were formed.
+++ indicates that in 50–72 per cent of the tests nodules were formed.
++ indicates that in 25–50 per cent of the tests nodules were formed.
+ indicates that in less than 25 per cent of the trials nodules were formed.
− indicates that no nodulation at all occurred.

Table 4 summarizes the preferences of a number of strains from wild leguminous plants for soybean, cowpea, and lupine plants; these data were obtained from at least four different greenhouse tests employing each of the host plants concerned.

Here, then, are a number of plants which, because bacteria isolated from their root systems will also form nodules on the cowpea plant, are considered as belonging to the cowpea cross-inoculation group. In addition to this ability to form nodules on *Vigna sinensis*, however, a good number of the cultures from these different hosts possess the ability to produce nodules on the soybean plant, and a few of them will also form nodules on lupine plants. In some of these strains, for example the Baptisia cultures, a preference for one host over another is evident. In others, like the Crotalaria and Lespedeza

cultures, no such specificity is observed, and a crossing over among soybean, lupine, and cowpea plants, and other closely-related hosts in the cowpea group, may occur. Further than this, certain cultures obtained from each of these three hosts have been shown, not only in the present studies but also in earlier reports (3, 15, 16, 20), to be capable of inducing nodule formation on the roots of each of the other species.

A cross-inoculation group is considered to be made up of a number of "plants among which the nodule-forming bacteria are mutually interchangeable." In accepting this definition and in evaluating these data upon the interrelationships of the plants and the organisms from what are now considered the soybean, cowpea, and lupine groups, it would seem that here is more evidence to support the consolidation of these now-separate groups into one, under a common name and subject to infection by a single species of Rhizobium.

If the observations presented in this paper represented all the data upon the interchangeability of the bacteria and the plants within the groups there would, perhaps, be no argument against the unification of the groups. As it is, however, there are recorded in the literature too many irregularities, too many instances of host specificities and of failures to cross, too many unexplained physical and chemical influences upon infection and nodule-formation in leguminous plants to permit of such a move at this time. It may be that an excess of caution has governed the decisions of recent workers in this controversial field, but it is felt that final pronouncement were best reserved until the weight of much more evidence has been built up for or against such a consolidation.

Perhaps eventually the solution to the problem will be found in the suggestion advanced by Allen and Allen (1), who state that "it is very likely that the cowpea group, now so very large and unwieldy as a unit in the sense of the present definition of a cross-inoculation group, could better be subdivided into smaller or sub-groups based on susceptibility to certain strains of rhizobia from plants more or less closely-related, and yet maintain some degree of unity." If, as they hold, the soybean plant would probably "bear a close relationship to certain ones of these (sub-) groups," these recent studies indicate that the lupine plant, too, will just as probably enter into the cowpea group-complex as the representative of another sub-group.

SUMMARY

A study was made of the morphological and cultural characteristics of root-nodule bacteria isolated from 44 species of wild leguminous plants found in Wisconsin. Classification of these cultures and of the plants from which they were obtained into cross-inoculation groups was attempted.

Ten new species are added to the cowpea group and six to the pea and vetch group, and one new species is added to the Robinia group and another to the Strophostyles group. Eleven species requiring further study are as yet

unassociated with any particular group and may possibly belong to new and distinct cross-inoculation groups.

Although evidence is presented which shows interrelationships of organisms and plants from the soybean, cowpea, and lupine groups, it is felt that this evidence and that obtained from reports by other workers is not yet sufficient to warrant the consolidation of these three groups into a single group under a common name and subject to infection by a common organism.

REFERENCES

(1) ALLEN, O N , AND ALLEN, E. K 1936 Root-nodule bacteria of some tropical leguminous plants· I Cross-inoculation studies with *Vigna sinensis* L *Soil Sci.* 42: 61–77.

(2) American Public Health Association 1920 Standard Methods for the Examination of Water and Sewage, ed 4 Boston

(3) CARROLL, W. R 1934 A study of Rhizobium species in relation to nodule formation on the roots of Florida legumes *Soil Sci* 37 117 135

(4) CONKLIN, M E 1936 Studies of the root-nodule organisms of certain wild legumes. *Soil Sci* 41 167–186

(5) FASSETT, N. C 1936 Notes from the Herbarium of the University of Wisconsin. XIII *Oxytropis chartacea* n sp *Rhodora* 38. 94

(6) FRED, E B., BALDWIN, I L , AND McCOY, E 1932 Root-nodule bacteria and leguminous plants Univ Wis Studies Sci 5

(7) FRED, E B , AND WAKSMAN, S A 1928 Laboratory Manual of General Microbiology McGraw-Hill, New York

(8) GRAY, ASA 1908 New Manual of Botany, ed 7 American Book Company, New York

(9) GRAY, P H H 1926 A method of staining bacterial flagella. *Jour Bact* 12 273–274

(10) ITANO, A , AND MATSUURA, A 1934 Nodule bacteria of *Astragalus sinicus* (Genge). II, III *Ber. Ōhara Inst Landw Forsch* 6 259, 341

(11) KOPELOFF, N , AND BEERMAN, P 1922 Modified Gram stains *Jour Infect Diseases* 31 480–482.

(12) KOSER, S A 1924 Correlation of citrate utilization by members of the colon-aerogenes group with other differential characters and with habitat *Jour. Bact* 9· 59–77

(13) LAIRD, D G. 1932 Bacteriophage and the root-nodule bacteria *Arch. Mikrobiol.* 3 159–193

(14) MÜLLER, A , AND STAPP, C. 1925 Beitrage zur Biologie der Leguminosenknöllchenbakterien mit besonderer Berucksichtigung ihrer Artverschiedenheit *Arb Biol. Reichsanst. Land u. Forstw* 14 455–554.

(15) RAJU, M. S. 1936 Studies on the bacterial-plant groups of Cowpea, Cicer, and Dhaincha I Classification *Centbl. Bakt.* (II) 94 249–262

(16) REID, J. J. 1936 The infective ability of rhizobia of the soybean, cowpea, and lupine cross-inoculation groups Ph. D. Thesis, Univ Wis

(17) SARLES, W. B., AND REID, J. J. 1935 Growth stimulants for rhizobia *Jour. Bact* 30 651

(18) Society of American Bacteriologists Manual of Methods for Pure Culture Study of Bacteria. Geneva, N. Y

(19) TATUM, E L., PETERSON, W. H , AND FRED, E. B 1934 An unknown factor stimulating the formation of butyl alcohol by certain butyric acid bacteria *Jour. Bact.* 27: 207–217.

(20) WALKER, R. H., AND BROWN, P. E. 1935 The nomenclature of the cowpea group of root-nodule bacteria. *Soil Sci.* 39: 221–225.

MICROBIOLOGICAL ASPECTS OF DECOMPOSITION OF CLOVER AND RYE PLANTS AT DIFFERENT GROWTH STAGES[1]

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Information on the decomposition of plant materials at different stages of maturity is important from the point of view of applying green manure to soils in agricultural practice. The value of organic matter in the soil has frequently been estimated from the rapidity with which the metabolic products, especially ammonia and nitrate, are formed. A microbiological study of decomposition of organic matter in the soil, involving a knowledge of the rapidity of the liberation of nitrogen in available forms during the process of decomposition, therefore, resolves itself into problems concerned with the chemical composition of the plant material added to the soil, the decomposition of the various plant complexes, and the nature of the organisms taking part in the decomposition process. The present investigation is concerned with the difference in the chemical composition of certain plants at different stages of maturity, the decomposition of these plants in soils of various moisture contents, and the influence of the plants upon the soil microflora.

The factors influencing the rate of decomposition of organic materials and the liberation of nitrogen in an available form have been the subject of many investigations. Only a few of the papers will be referred to here. In 1897, Wollny (27) demonstrated that the higher the nitrogen content of certain plant materials the more rapid is their decomposition in soils. Following the work of Wollny, much consideration has been given to the relation of the nitrogen content and the C/N ratio of organic materials upon the rate of decomposition of these materials. Rahn (12) studied the effects of the presence of available nitrogen on straw decomposition and found that nitrate or ammonia nitrogen accelerated the decomposition of straw 2 days after its application to the soil. The straw-treated soil normally became deficient in nitrogen, but legume-treated soils did not show this effect. Among other workers, Waksman (18), Starkey (16), Whiting (24), and Jensen (6) have found that organic materials with wide C/N ratios decompose slowly and that soils treated with such materials become deficient in available nitrogen. The plant constituents decompose in the soil more rapidly when the plant is at an early stage of growth than when it is at a later stage or when it begins to mature. Where

young plant material is decomposing in the soil the nitrogen is liberated most rapidly in an available form, namely, ammonia, which is changed to nitrate. Maynard (10) and Merkle (11) found that the younger the crop turned under, the more rapidly does it decompose in the soil. By determining the carbon dioxide evolution as an index of decomposition, Hill (4) demonstrated not only that plants at an early stage of growth decompose most rapidly but that a definite relation exists between the nitrogen content of the plant and its decomposition: the higher the nitrogen content the more rapidly does the plant material decompose. Waksman and Tenney (23) made a detailed study of the decomposition of rye plants at different stages of growth and found that the rapidity of decomposition of young plants depends upon the amount of water-soluble constituents and upon the nitrogen content. Variation in the composition of plants at different stages of growth has been correlated with the rate of decomposition by Leukel, Barnette, and Hester (7), who found a progressive decrease in the percentage of cellulose and lignin as the plants advanced toward maturity; rapidity of nitrification of the complete plants decreased with successive growth stages. According to Waksman (19), an abundance of water-soluble constituents, a high protein content, and a low hemicellulose, cellulose, and lignin content lead to rapid disintegration of young plants and rapid liberation of the nitrogen. On the other hand, a low content of water-soluble constitutents, a high content of cellulose, hemicellulose, and lignin, and a low protein content lead to slow decomposition of mature plants and plant residue. Similar results have been obtained by more recent investigators (1, 17, 26).

Considerable attention has been devoted to the effects of decomposition of organic matter on the soil microflora, but information on the decomposition of organic materials of various growth stages is more meager. It is sufficient to cite the results of Engberding (3), who added both green rye and vetch to the soil and found that the plate count of microorganisms increased at the end of 7 weeks approximately 500 per cent with rye and nearly 400 per cent with vetch. At the end of 10 weeks the numbers dropped considerably. Briscoe and Harned (2), using alfalfa, oats, and cowpeas for green manure, found a direct relationship between the total plate count of soil microorganisms and the amount of organic matter added. Waksman and Starkey (21) demonstrated that rye straw and alfalfa greatly influenced the development of microorganisms in the soil; alfalfa increased the numbers of both bacteria and fungi to a greater extent than did rye straw. Martin (8, 9) found that the nature of the organic materials incorporated in the soil determines the nature of the fungi developing in the soil. He also demonstrated that alfalfa and sweet clover roots added to the soil influence the development of fungi less than do the tops.

EXPERIMENTAL METHODS

For studies on the decomposition of plant materials of various growth stages, abruzzi rye (*Secale cereale*) and crimson clover (*Trifolium incarnatum*)

were selected. The clover and rye seeds were planted in the fall adjacent to each other on a plat of ground of uniform soil type which had received no special fertilizer treatment. The plants used in the experiment were selected at random from various parts of the plat and were well mixed. The first sample of clover (young clover) was taken when the plants were 8 to 10 inches tall before the flower buds had begun to form; the second sample (medium clover) was taken just after the plants were in full bloom; and the third sample (mature clover), after the plants had reached maturity as indicated by the leaves turning brown and the seeds becoming hard. The first sample of rye (young rye) was taken when the plants were 10 to 14 inches tall, before any of the heads had begun to form; the second sample (medium rye) was taken when the heads of the plants were well formed but none of the plants were in bloom; and the third sample (mature rye) was taken after the plants had reached maturity as indicated by the seed in the hard dough state. After the plants were cut, they were immediately dried, and the seeds were removed from the mature plants. All samples were ground, and the fresh material was analyzed for the following constituents: ether- and alcohol-soluble fractions, cold- and hot-water-soluble organic matter, hemicellulose, cellulose, lignin, crude protein, ash, and total nitrogen. The method recommended by Waksman and Stevens (22) was used in the analysis.

For the study of the processes of decomposition and nitrogen transformation in soils of different moisture contents, the evolution of carbon dioxide (20) and the formation of ammonia and nitrates were determined. Two sections containing 10 units each of the aeration apparatus were used in the experiment. A course sandy loam soil was air dried and put through a 3-mm. sieve. One-hundred-gram portions of the air-dried soil and one-gram portions of the ground material were thoroughly mixed and placed in 300-cc. flasks. Distilled water was added to each pair of flasks to bring the moisture contents to 9, 13.5, 18, 22.5, and 27 per cent. The flasks were connected with the aeration apparatus and kept in the laboratory at room temperature. The ammoniacal nitrogen was determined on an amount of soil equivalent of 25 gm. from each flask; the ammonia was leached out with a normal solution of potassium chloride and distilled into standard acid, heavy magnesium oxide being used. For the nitrate nitrogen the phenoldisulfonic acid method was used. The carbon dioxide was collected for 28 days and calculated on the basis of 7-day intervals, and the ammonia and nitrate were determined at the end of 7, 14, 21, and 28 days. The process was repeated until the six samples of material were studied.

COMPOSITION OF PLANT MATERIAL

The composition of the plants at different stages of growth is recorded in table 1. The results, stated as averages of duplicate samples, show great differences in the composition of the young, the medium, and the mature plants. As the plants advanced toward maturity, the percentages of ether- and alcohol-soluble fractions, water-soluble fractions, ash, and crude pro-

tein decreased progressively, and the percentages of hemicellulose, cellulose, and lignin increased progressively.

In the young rye and clover plants, the water-soluble constituents make up 33 and approximately 40 per cent of the total constituents respectively. In the same plants, the crude protein was considerably higher than in the mature plants. On the other hand, the mature rye and clover contained greater amounts of the more complex constituents, cellulose and lignin, than did the younger plants.

A comparison of the composition of the two types of plants of the same age, clover and rye, shows that the clover contained more of the water-soluble fraction, ether- and alcohol-soluble fractions, ash, and crude protein, than did the rye, whereas the rye contained more cellulose, hemicellulose, and lignin. The total nitrogen decreased from 3.65 per cent in the young rye to

TABLE 1

Proximate chemical composition of rye and clover plants at different stages of growth

CONSTITUENT	RYE			CLOVER		
	Young	Medium	Mature	Young	Medium	Mature
	per cent	per cent	per cent	per cent	per cent	per cent
Ether- and alcohol-soluble fraction	6.55	3.87	2.47	7.03	4.56	4.31
Cold- and hot-water-soluble organic matter...	33.00	21.12	19.11	40.64	31.08	24.57
Hemicellulose	13.51	17.27	20.40	8.11	10.80	12.12
Cellulose..	21.56	30.96	36.30	16.70	28.40	31.27
Lignin	3.98	7.20	11 33	3.82	5.68	8 36
Crude protein	10.55	6 97	2.37	11.67	8.70	4.91
Ash........	8.75	7.70	3.56	10.60	8 38	8.20
Total..... ..	97.90	95.09	95.54	98.57	97.60	93.74

0.68 per cent in the mature rye, whereas it decreased from 3.15 per cent in the young clover to 1.49 per cent in the mature plant. Although the young rye contained more total nitrogen than did the young clover, the former contained less crude protein. The nitrogen content of the rye plants decreased much more rapidly than did that of the clover as the plants advanced toward maturity. This is important from the point of view of adding crop residue and other organic matter to the soil and probably accounts for the fact that residues from legume crops liberate more available nitrogen than do residues from non-legumes when decomposed in the soil.

INFLUENCE OF MOISTURE ON THE DECOMPOSITION OF PLANT MATERIALS

For the study of the influence moisture on decomposition, the clover and rye plants harvested at different stages of growth were used. The moisture content of the soil was adjusted to 9, 13.5, 18, 22.5, and 27 per cent. The

influence of the various moisture concentrations on the rate of decomposition
was not so pronounced as might have been expected.   The effects were paral-
lel with both plants in all stages except the mature state.   Decomposition was
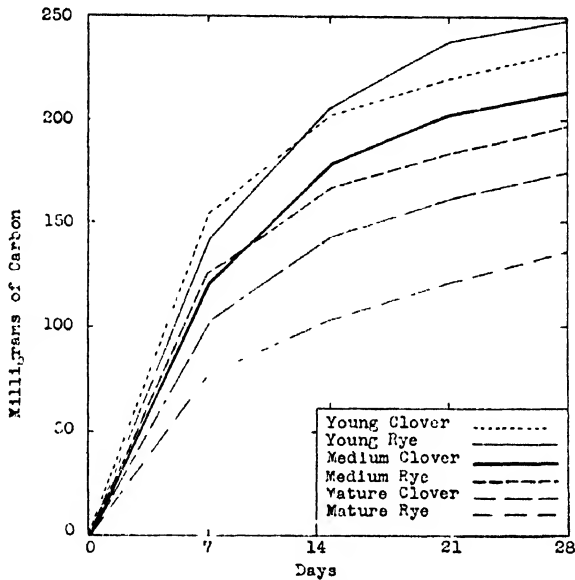
FIG. 1. COURSE OF EVOLUTION OF CARBON DIOXIDE FROM RYE AND CLOVER PLANTS IN SOIL
CONTAINING 18 PER CENT MOISTURE
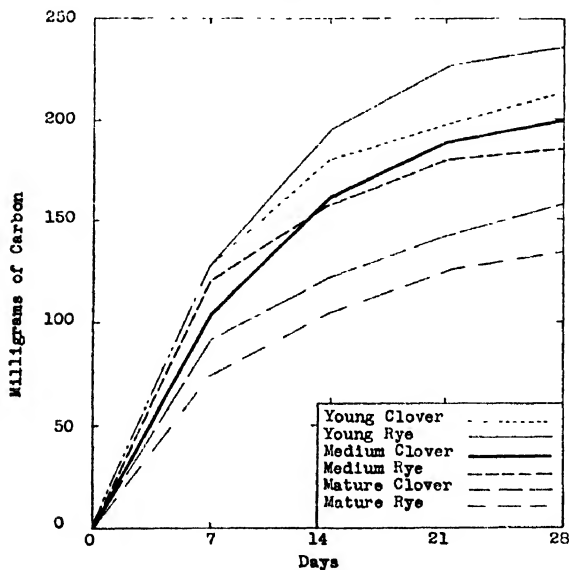
FIG. 2. COURSE OF EVOLUTION OF CARBON DIOXIDE FROM RYE AND CLOVER PLANTS IN SOIL
CONTAINING 27 PER CENT MOISTURE

slowest in the soil containing the lowest, 9 per cent, and the highest, 27 per cent, moisture content. Decomposition was favored in soils up to 18 per cent moisture concentration, as indicated by the evolution of carbon dioxide. The course of decomposition of the various materials in soil with 18 per cent moisture is shown in figure 1, and that in soil with 27 per cent moisture is shown in figure 2. A comparison of the two figures shows that decomposition is somewhat slower in the soil with the higher moisture concentration. The young plants decomposed much more rapidly than did the medium or mature plants. The difference in the rate of decomposition can be explained on the basis of the chemical composition of the plants. The young plants, containing more water-soluble constituents, decomposed more rapidly than did the mature plants, which also contained less than half the amount of soluble nitrogen present in the young plants. The less resistant constituents of the plants are more easily attacked by the microorganisms for energy and cell synthesis, and the more resistant constituents resist the attack by microorganisms and persist for a longer time.

A comparison of the rates of decomposition of plants of different nature (clover and rye), as suggested by Whiting and Richmond (25), indicates that soluble nitrogen exerts the most pronounced effect. The young clover contained a higher percentage of water-soluble constituents than did the young rye but lagged behind the rye in the amount of carbon dioxide produced. The soluble nitrogen content of the young clover and rye was 15.56 mgm. and 21.4 mgm. respectively. This probably explains the difference in the decomposition of the two plants of the same age. The medium clover contained more soluble nitrogen (13.06 mgm.) than did the medium rye (12.0 mgm.); the former decomposed more rapidly than the latter. The mature clover, containing 7.09 mgm. soluble nitrogen, decomposed more rapidly than did the mature rye, which contained only 3.6 mgm. soluble nitrogen.

NITROGEN TRANSFORMATION

The liberation of ammonia and nitrate was greatly influenced by the moisture content of the soil, by the composition of the organic material, and by the period of incubation.

The nitrate nitrogen increased with increase in the concentration of the soil moisture up to 18 per cent and decreased at moisture concentrations of 22.5 and 27 per cent, and higher moisture concentrations affected the formation of ammonia to a limited extent. The amounts of nitrogen liberated in the presence of 22.5 and 27 per cent moisture agree with the findings of other investigators (5, 15) that nitrate formation is depressed and ammonia formation is either unaffected or increased in the presence of high moisture content. The course of nitrogen transformation from the various materials in soils with 18 per cent moisture is shown in figure 3, and that of the nitrogen transformed in soil with 27 per cent moisture is shown in figure 4. A comparison of these figures readily shows that the concentration of the soil moisture has a marked

influence on the amount of nitrogen liberated in the decomposition of plant materials.

The composition of the plants influenced the liberation of both ammonia and nitrate. The greatest amount of nitrate was liberated from the young clover, and the greatest amount of ammonia, from the young rye. The young
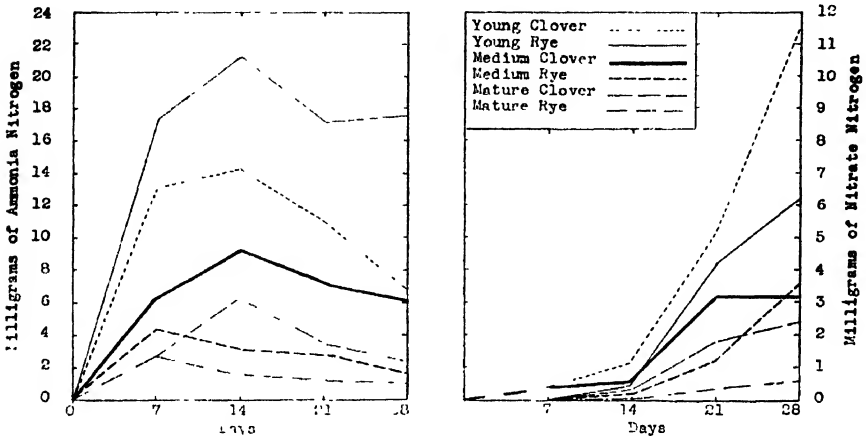
FIG. 3. COURSE OF TRANSFORMATION OF NITROGEN FROM RYE AND CLOVER PLANTS IN SOIL CONTAINING 18 PER CENT MOISTURE
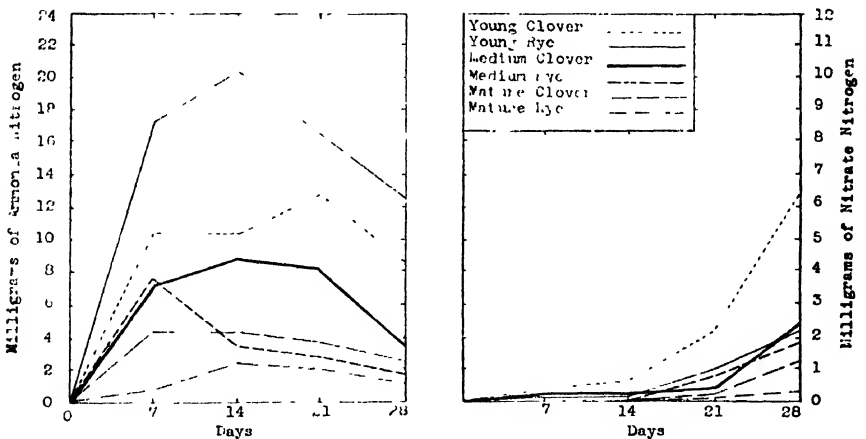
FIG. 4. COURSE OF TRANSFORMATION OF NITROGEN FROM RYE AND CLOVER PLANTS IN SOIL CONTAINING 27 PER CENT MOISTURE

rye contained a higher content of total nitrogen and water-soluble nitrogen than did the young clover, but less of the nitrogen content of young rye than that of the medium clover was transformed into nitrate. The slower transformation of the nitrogen into nitrate from the rye than from the clover might be due to the nature of the nitrogen in the rye plant. The results clearly

show that a close relationship exists between the chemical composition of natural organic materials and the rate of nitrogen transformation during decomposition. Young plants with a high protein content and a narrow C/N ratio are transformed with a rapid liberation of the nitrogen. On the other hand, the nitrogen of mature plants with a low nitrogen content and a wide C/N ratio is not liberated rapidly. This explains why less nitrate was formed in the presence of mature plants than in the control soil, even after a period of 28 days: the nitrogen content of the plants was too low to meet the requirement of the microorganisms, and even the soil nitrogen was consumed by the microorganisms to satisfy their requirement for the decomposition of the plant material. During the first 14 days of decomposition less nitrate was formed in soils containing plant materials, except young clover, than in the controls. At the end of 7 days, an insignificant amount of nitrate had formed from the mature plants. Evidently the nitrogen liberated during the most active period of decomposition was assimilated by the microorganisms for cell synthesis. At the end of 21 days, decomposition was less active than it had been, and nitrate accumulated in excess of the control from the young rye as well as from the young and medium clover plants. At the end of 28 days, the period of rapid decomposition had been completed, the C/N ratio had become narrow, and considerable nitrate had accumulated in excess of the control from all but the mature plants.

Ammonia was liberated from all plants during the first 14 days of decomposition; after that, active nitrification set in, so that at the end of 28 days the ammonia had decreased considerably. Evidence indicates that the ammonia was not nitrified during the most active period of decomposition but that as soon as the active period ceased it was transformed into nitrate. Richards and Shrikhande (13) suggested that during the break-down of straw the microorganisms concerned have a definite preference for ammonia over nitrate. The results here indicate that the nitrate progressively increases and the ammonia progressively decreases with the period of incubation. One must also consider, of course, the injurious effect of rapidly decomposing organic substance upon the activities of the nitrifying bacteria.

INFLUENCE OF THE DECOMPOSITION OF CLOVER AND RYE PLANTS ON THE SOIL MICROFLORA

Further studies were undertaken to obtain some idea of the nature of the microorganisms which take an active part in the decomposition of various organic materials and the effect of the materials on the microbiological population of the soil. The materials described previously were used in these studies: young, medium, and mature clover and rye plants of corresponding ages.

Duplicate 5-gm. portions of the dry samples of the organic materials were thoroughly mixed with 1-kgm. portions of Sassafras loam of pH 6.4. The soils were placed in glazed pots, and the moisture was adjusted to 60 per cent saturation and kept at that point during the course of the experiment. The

pots were covered with glass and incubated at room temperature in the laboratory. Two pots of the soil without organic matter served as controls. The numbers of microorganisms in the soils were determined 0, 5, 10, 20, 30, and 60 days after the addition of the organic materials. The abundance of cellulose-decomposing bacteria was determined after a period of 30 days. The common plate method, the direct microscopic method, and the dilution method were used for determining the numbers. Bacteria and actinomyces were determined together on albumin agar, and the fungi were determined on acid agar. Numbers of cellulose-decomposing bacteria were determined from growth in tubes of a solution medium containing strips of filter paper inoculated with dilutions of the soil. After each sample was removed from the pots for the determination of numbers of microorganisms, a microscope slide was placed in each pot and allowed to remain in contact with the soil for a period of 48 hours. The slides were then removed, dried, and stained, and the types of microorganisms that had developed upon the slides were studied under the microscope.

Influence of the organic materials on the abundance of microorganisms

At the time the experiment was started the number of microorganisms was greater in the untreated soil than in the treated soil. Small pieces of organic materials containing no microorganisms were present in the soil samples and probably account for this difference. The data presented in figure 5 show that the peak of development of bacteria and actinomyces was reached 5 days after the addition of the organic materials and that the numbers decreased during the subsequent incubation period for all the materials. The number of bacteria and actinomyces in the soil treated with young clover showed an increase of 155,000,000 over that of the control soil. The medium and mature clover influenced the development of bacteria and actinomyces somewhat less; the former increased the number 124,000,000 and the latter 67,000,000 over the control soil in 5 days. The rye plants also increased the number of bacteria and actinomyces, the greatest increase occurring in the soil receiving the young plants. A greater increase in the number of bacteria and actinomyces resulted from the clover plants than from the rye plants of corresponding ages throughout the experiment.

The chemical composition of the plants determined the extent of microbial development. The young plants contained a large amount of water-soluble organic matter and of nitrogen; the older plants were rich in resistant organic substances and were low in nitrogen. The soluble organic matter is decomposed rapidly, and in the young plants there is no nitrogen deficiency. In the mature plants a large part of the organic matter was resistant to decomposition, and the quantity of nitrogen was insufficient to satisfy the nutritional requirement of the microorganisms. The decrease in the number of bacteria and actinomyces after the 5-day period was associated with the transformation of the available microbial food contained in the material. The rapid develop-

ment of bacteria and actinomyces during the early incubation period exhausted the supply of most available food, resulting in a subsequent decrease in the numbers.

Although Smith and Humfeld (14) reported that turning under green rye and vetch in the soil produces very little change in the number of fungi, the data presented in figure 6 show that the younger the plant, the greater is
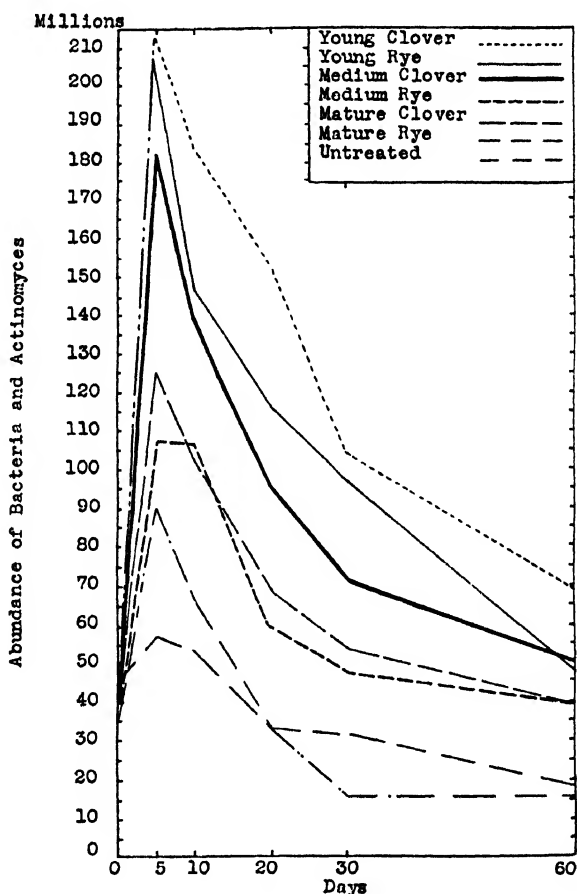
FIG. 5. INFLUENCE OF RYE AND CLOVER ON THE DEVELOPMENT OF BACTERIA AND ACTINOMYCES IN SOILS

the development of fungi. The number of fungi increased twelvefold in the soil treated with young clover over that in the untreated soil. The medium and mature clover also increased the number of fungi but to a lesser degree than did the young clover plants. Although the rye plants increased the number of fungi to a marked degree, the change was not so great as that caused by the clover plants of corresponding ages. The number of fungi increased

tenfold in the soil treated with young rye plants over that in the untreated soil; medium and mature rye had less effect upon the development of fungi than did the young rye. Although the peak of the growth of bacteria and actinomyces occurred in the soil 5 days after the addition of the organic materials, the peak of development of fungi was not reached until 20 days after the organic materials were added to the soil, and the number of fungi decreased during the remainder of the incubation period. The rate of decrease, however, was not the same in all soils. The numbers dropped somewhat more rapidly in the soils that contained the younger plants. This may have been due to the more rapid exhaustion of the available organic matter in the young plants.
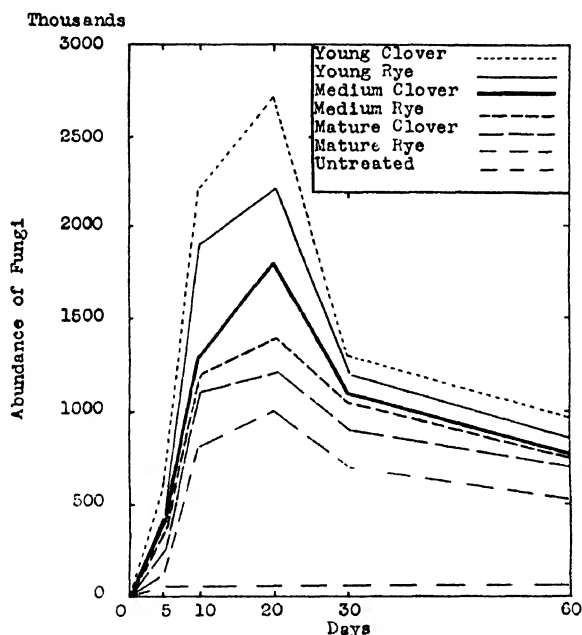
FIG. 6. INFLUENCE OF RYE AND CLOVER ON THE DEVELOPMENT OF FUNGI IN SOILS

The numbers of cellulose-decomposing bacteria were determined after decomposition had progressed for 30 days. The results are presented in table 2. It is of interest that the number of cellulose-decomposing bacteria can be correlated with the amount of cellulose decomposed during the process of composting.[2] The soil treated with young rye gave growth of cellulose-decomposing bacteria at the highest dilution, 100,000,000; during the process of composting, more cellulose disappeared from the young rye plant than from either the medium or the mature plant. The soil treated with the mature rye plants yielded no greater number of bacteria than did the untreated soil, 10,000. Less of the cellulose in the mature rye plants decomposed than

[2] Unpublished data.

in the young or medium plants. The soil treated with the young clover plants gave growth in the ten-millionth dilution; the amount of cellulose that decomposed during the process of composting was less than the amount decomposed in the young rye plant.

Direct microscopic counts of the microorganisms in the soils showed no consistent relationship to plate counts. The results of the direct microscopic counts presented in table 3 show that there were much larger numbers of microorganisms in the soil than were estimated by the plate count.

TABLE 2

Influence of organic matter on the development of cellulose-decomposing bacteria

SOIL TREATMENT	DILUTION				
	10,000	100,000	1,000,000	10,000,000	100,000,000
Young clover.........................	X	X	—	X	—
Medium clover.............	X	X	—	—	—
Mature clover.................... ..	X	X	X	—	—
Young rye..................... . ..	X	X	—	X	X
Medium rye..........	X	X	—	—	—
Mature rye..................... ..	X	—	—	—	—
Untreated...........	X	—	—	—	—

X indicates growth; —, no growth.

TABLE 3

Direct microscopic count of bacteria in soil treated with some organic substances

Millions of bacteria per gram of soil

SOIL TREATMENT	BACTERIA AFTER INCUBATION OF					
	0 days	5 days	10 days	20 days	30 days	60 days
Young clover plant....	401	1,923	1,742	1,621	1,136	703
Medium clover plant............... ..	376	1,620	1,503	897	6,079	511
Mature clover plant.................	352	1,141	978	702	560	396
Young rye plant................... ..	388	1,763	1,481	1,178	987	461
Medium rye plant....................	311	972	879	593	483	351
Mature rye plant....................	321	812	681	287	261	169
Untreated...........................	481	601	634	482	187	162

Influence of the organic materials on the types of microorganisms

Examination of the slides kept in contact with the soil during part of the period of decomposition revealed that the types of microorganisms which developed were influenced by the kind of organic matter added to the soil as well as by the extent of the decomposition of the materials. During the initial decomposition of the young clover, the bacteria were predominantly aggregates of coccoid cells. There were also a few short rods and small irregularly shaped cells. No coccoid cells were observed on the slides in contact with

soils treated with young rye, but chains of short rods and many isolated small cells were detected. Slides in contact with soil treated with medium clover showed mostly short rods, groups of small cells, and a few long rods. The types of organisms developing on the slides in contact with soils treated with medium rye were principally isolated short rods and long cells. The soil treated with the mature plants developed somewhat different types of organisms. In the soil treated with clover, there were large long cells, short rods, and a few oval cells; in the soil treated with rye, short rods, long thin rods, and large oval cells were noted. As decomposition progressed, the types of organisms changed. In the latter part of the decomposition period, the types of organisms encountered on the slides were not so variable as those that appeared during the early part of the decomposition period and were principally rods either isolated or in aggregates. Long thin rods, long thick rods, short rods, and spiral-shaped cells were observed on the slides in the latter part of the decomposition period. The long thick rods were observed on the slides in contact with the soils treated with the clover plants, and the long thin rods were observed on slides in contact with the soils treated with rye plants. Coccoid cells were observed on the slides in contact with soils treated with young clover during the entire course of the decomposition period.

The types of fungi that developed in the soil differed somewhat in response to the various treatments. Penicillium, Mucor, Fusarium, and Trichoderma developed on acid agar plates from the soil treated with the rye plants. Penicillium was the dominant group on plates from the soil that was treated with mature rye plants and developed in greater numbers from this soil than from the other soils. Mucor, Fusarium, and Trichoderma occurred in about the same proportion on the plates from the soils treated with the rye plants of different ages. A larger number of generic groups of fungi was found in the soils treated with the clover plants than in the soils treated with the rye plants. The predominating genera were Penicillium, Mucor, Aspergillus, Fusarium, Trichoderma, Thamnidium, and Cunninghamella. Aspergillus was recovered principally from the soil treated with young clover; Thamnidium and Cunninghamella were obtained only from the soil treated with medium and mature clover.

SUMMARY

Studies were made on the microbiological aspects of decomposition of rye and clover plants at different stages of maturity.

The chemical composition of the plant undergoes marked changes during plant growth.

Young plants contain a larger proportion of fats and other ether- and alcohol-soluble substances, carbohydrates, proteins, and soluble ash. As the plants advance toward maturity, these constituents progressively decrease, and such constituents as cellulose, hemicellulose, and lignin progressively increase.

The rate of decomposition of organic materials depends upon the composi-

tion of the plant. The higher the concentration of water-soluble constituents, especially the carbohydrate and the nitrogen fractions, the more rapid is the rate of decomposition.

The moisture content most favorable for maximum decomposition appears to be about 18 per cent. Decomposition is retarded when the moisture content is as low as 9 per cent or as high as 27 per cent.

Nitrogen transformation of organic material is influenced by the chemical composition of the material and by the moisture content of the environment. The liberation of nitrate in the soil is depressed at a moisture content above 18 per cent.

When organic matter is undergoing decomposition in the soil, nitrate is not liberated for a period of 21 to 28 days, or until the active period of decomposition is complete. Ammonia accumulates in greatest amounts during the early part of the active period of decomposition and remains virtually constant until the rate of decomposition decreases; the ammonia then changes into nitrate.

The abundance of soil microorganisms, including bacteria, actinomyces, and fungi was greatly increased by all the plant materials, the extent of the increase being determined by the nature of the organic material.

The young plants increased the number of microorganisms to a greater extent than did the mature plants; the clover plants favored the development of a more abundant population than did the rye plants. Both plate counts and direct microscopic count reflected these changes.

The largest number of cellulose-decomposing bacteria was detected in the soil treated with young rye, the next greatest number in the soil receiving young clover. No greater number of cellulose bacteria was found in the soil with mature rye than in the untreated soil.

The composition of the plant material, differing with the species and stage of maturity, determines the type of microorganisms which will participate in the decomposition process.

Different groups of fungi developed in response to the various materials.

REFERENCES

(1) ALBRECHT, W. A., AND ALLISON, W. H. 1931 Changes in composition of soybeans toward maturity as related to their use as green manure. *Soil Sc.* 32: 271–282.

(2) BRISCOE, C. F., AND HARNED, H. H. 1915 Bacteriological effects of green manure. Miss. Agr. Exp. Sta. Bul. 168.

(3) ENGBERDING, D. 1909 Vergleichende Untersuchungen über die Bakterienzahl im Ackerboden in ihre Abhängigkeit von Susseren einflussen. *Centbl. Bakt.* (II) 23: 569–642.

(4) HILL, H. H. 1926 Decomposition of organic matter in soils. *Jour. Agr. Res.* 33: 77–79.

(5) JANSEN, G., AND METZER, W. H. 1928 Transformation of nitrogen in rice straw. *Jour. Amer. Soc. Agron.* 20: 459–476.

(6) JENSEN, H. L. 1929 On the influence of the carbon nitrogen ratios of organic materials on the mineralization of nitrogen. *Jour. Agr. Sci.* 19: 71–82.

(7) LUEKEL, W. A., BARNETTE, R. M., AND HESTER, J. B. 1929 Composition and nitrification studies on Crotalaria striata. *Soil Sci.* 28: 347–371.

(8) MARTIN, T. L. 1927 Decomposition studies of alfalfa and sweet clover roots and straw. *Soil Sci.* 24: 309–315.

(9) MARTIN, T. L. 1929 The effect of sweet clover and alfalfa roots and tops on the fungus flora of the soil. *Soil Sci.* 27: 399–405.

(10) MAYNARD, L. A. The decomposition of sweet clover as green manure under green house conditions. N. Y. (Cornell) Agr. Exp. Sta. Bul. 394: 119–149.

(11) MERKLE, F. G. 1918 The decomposition of organic matter in soils. *Jour. Amer. Soc. Agron.* 10: 281–302.

(12) RAHN, O. 1919 Die schädliche Wirkung der Ströhdüngung und deren Verhütung. *Ztschr. Tech. Biol.* 7: 172–186.

(13) RICHARDS, E. H., AND SHRIKHANDE, J. G. 1935 The preferential utilization of different forms of inorganic nitrogen in the decomposition of plant materials. *Soil Sci.* 39: 1–8.

(14) SMITH, N. R., AND HUMFELD, H. 1930 Effect of rye and vetch green manures on the microflora, nitrates and hydrogen-ion concentration of two acid and neutralized soils. *Jour. Agr. Res.* 41: 97–123.

(15) SREENIVASAN, A., AND SUBRAHMANYAN, V. 1935 Biochemistry of water-logged soils. IV. Carbon and nitrogen transformation. *Jour. Agr. Sci.* 25: 6–21.

(16) STARKEY, R. L. 1924 Some observations on the decomposition of organic matter in soils. *Soil Sci.* 17: 293–314.

(17) TURK, L. M. 1932 The composition of soybean plants at various growth stages as related to their rate of decomposition and their use as green manure. Missouri Agr. Exp. Sta. Res. Bul. 173.

(18) WAKSMAN, S. A. 1924 Influence of microorganisma upon the carbon nitrogen ratio in soil. *Jour. Agr. Sci.* 14: 555–562.

(19) WAKSMAN, S. A. 1929 Chemical and microbiological principles underlying the decomposition of green manure in soils. *Jour. Amer. Soc. Agron.* 21: 1–19.

(20) WAKSMAN, S. A., AND STARKEY, R. L. 1924 Microbiological analysis as an index to soil fertility: VII. Carbon dioxide evolution. *Soil Sci.* 17: 141–158.

(21) WAKSMAN, S. A., AND STARKEY, R. L. 1924 Influence of organic matter upon the development of fungi, actinomycetes and bacteria in soils. *Soil Sci* 17: 373–378.

(22) WAKSMAN, S. A., AND STEVENS, R. 1930 A system of proximate chemical analysis of plant materials. *Indus. and Engin. Chem., Analyt. Ed.* 2: 167–183.

(23) WAKSMAN, S. A., AND TENNEY, F. G. 1927 The composition of natural organic materials and their decomposition in the soil: II. Influence of age of plant upon the rapidity and nature of its decomposition—rye plants. *Soil Sci.* 24: 317–333.

(24) WHITING, A. L. 1926 Some important factors controlling the rate of nitrification of organic materials. *Jour. Amer. Soc. Agron.* 18: 854–876.

(25) WHITING, A. L., AND RICHMOND, T. E. 1927 The relative rate of nitrification of different parts of the sweet clover plants. *Soil Sci.* 24: 31–37.

(26) WINNIK, M. 1935 Composition of lupines at different stages as related to its decomposition in soils. *Trans. Third Internatl. Cong. Soil Sci.* 1: 226–229.

(27) WOLLNY, E. 1897 Die Zersetzung der organischen Stoff und die Humusbildungen Rücksicht auf die Bodencultur. Carl Winter's, Hiedelberg.

INFLUENCE OF ARTIFICIAL IRRADIATION UPON THE OXIDATION OF AMMONIA AND FORMATION OF NITRATE IN SOIL[1]

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It has been demonstrated (6) that exposure of soil to ordinary sunlight in temperate regions during the summer months does not result in any appreciable oxidation of ammonia, whether produced in the soil by the decomposition of organic matter or added to the soil. Any formation of nitrate in soil for which positive evidence was obtained was a result of biological activities only.

A series of experiments were now undertaken for the purpose of obtaining information on the influence of artificial illumination upon the processes of oxidation of ammonia and reduction of nitrate in soil. A high-pressure, water-cooled, vertical capillary mercury vapor lamp (3, 5) was run on a 500-volt D.C. line, started with 10 amperes passing through it and when burning using about 3.2 amperes, with about 300 volts across the terminals. The emitting column was 1 by 15 mm., giving per unit surface about 15 times the radiation of a commercial mercury vapor lamp. The radiation from the vertical lamp was made by means of a quartz condensing lens and a quartz reflection prism to cover a horizontal area of about 2 by 8 mm. The lamp produced radiation from 2,000 to 9,000Å, but since the beam passed through tap water, a fused quartz window, lens, and reflection prism, it is doubtful whether there was any radiation below 2,200Å. In some cases, a uviol glass filter was introduced, cutting out most of the radiation below 2,800Å.

In the first experiment, the samples of soil adjusted to optimum moisture were placed in a Petri dish so that the beam of light, 8 x 2 mm., would cover it; the soil was stirred every 15 minutes, all clumps being crushed, and moistened with distilled water. In the second experiment, the soil was made to cover an area with a diameter of 24 mm. on the bottom of a Petri dish, and the beam of radiation coming from the reflection prism covered an area of about 12 x 2 mm. The dish was rotated, at the rate of about two to three rotations per second. The soil was mixed every 10–15 minutes, all clumps being crushed, and sufficient distilled water was added to keep it moist. Because of the long irradiation period in this experiment, the temperature could not be kept low and occasionally reached a maximum of 40°C.

A light Sassafras sandy loam was used in all the experiments.   The samples of soil were prepared and shipped in sterile glass containers to Madison, Wisconsin, where they were submitted to the radiation treatment for a period of 2 hours with the full arc.   Both the untreated and the treated samples were then returned to New Brunswick, where they were subjected immediately to chemical and microbiological analyses.   In the first experiment, two lots of soil were prepared: 1, air-dried soil, and 2, fresh soil containing an optimum amount of moisture for biological processes.   Each lot was divided into three portions: (*a*) with no addition of nitrogen, (*b*) with addition of $(NH_4)_2SO_4$ +

TABLE 1

Influence of artificial light upon the oxidation of ammonium salt and upon nitrate reduction in soil

Salts added in amounts equivalent to 25 mgm. of nitrogen per 100 gm. of soil

SALT ADDED	EXPOSURE TO LIGHT	$NH_4 - N$ IN 100 GM. OF SOIL	$NO_3 - N$ IN 100 GM. OF SOIL	$NO_2 - N$	FUNGI, COLONIES PER PLATE*
	hours	mgm.	mgm.		
Air-dry soil					
None	0	3	1.47	Tr.	9, 5, 10
$(NH_4)_2SO_4$ + $CaCO_3$† ..	0	25	1.25	+	.. .
$NaNO_3$.	0	3	30.00	−	. .
None .	2	2	1.19	Tr.	1, 1, 2
$(NH_4)_2SO_4$ + $CaCO_3$.	2	22	1.19	+	. . .. ..
$NaNO_3$. ..	2	3	30.00	Tr.	. .. ..
Moist soil					
None.........	0	2	4.16	+	14, 15, 12
$(NH_4)_2SO_4$ + $CaCO_3$	0	24	5.30	++	12, 15. 9
$NaNO_3$	0	5	30.00	+	24, 30, 31
None	2	2	2.97	+	7, 21, 17
$(NH_4)_2SO_4$ + $CaCO_3$.	2	23	2.94	++	2, 3, 2
$NaNO_3$.	2	4	30.30	+	2, 2, 5

* Dilution 1:5,000 for air-dry soil and 1:10,000 for moist soil.

† 300 mgm. per 100 gm. of soil.

$CaCO_3$, and (*c*) with addition of $NaNO_3$.   The different forms of nitrogen were added at the rate of 25 mgm. per 100 gm. of soil.   The soil samples were prepared on October 20, irradiated on October 26, and analyzed on October 30.

The results presented in table 1 show that a slight decrease in ammonia occurred as a result of irradiation, both in the air-dry and in the moist soil; this was not accompanied, however, by any increase in nitrite or nitrate.   Whereas no biological oxidation of ammonia took place in the air-dry soil, oxidation was rapid in the moist soil, as shown by an increase in nitrate content with and without addition of ammonium salt.   The irradiation of the soil seemed to

have an injurious effect upon the nitrifying flora, as shown by the reduction in the amount of nitrate formed in the irradiated soil. This injurious effect was also evident from the numbers of fungi and bacteria, which were depressed by the treatment. In view of the fact, however, that several days elapsed between the irradiation treatment and the plating of the soil, there was ample opportunity for various organisms to make rapid growth upon the irradiated soil, which would tend to make the results somewhat erratic; hence they are not reported here in detail, and only the numbers of fungus colonies found on

TABLE 2

Effect of full arc and of filtered light upon the oxidation of ammonium salt and the reduction of nitrate in soil

Salts added in amounts equivalent to 25 mgm. of nitrogen per 100 gm. of soil

SALT ADDED	EXPOSURE TO LIGHT	$NH_4 - N$ IN 100 GM. OF SOIL	$NO_2 - N$ IN 100 GM OF SOIL	$NO_2 - N$	NITRIFYING BACTERIA*	FUNGI, COLONIES PER PLATE
		mgm.	*mgm*			
Control	None	2	4.16	0	+	7, 8, 11
$(NH_4)_2SO_4 + CaCO_3$†	None	26	5 20	Tr.	++++	12, 4, 11
$(NH_4)_2SO_4 + CaCO_3 + ZnSO_4$‡ + $MnSO_4$‡	None	28	5.20	+++	++++	12, 9, 6
$NaNO_3$	None	5	30.00	++	+++	12, 10, 10
Control	Full arc	7	2.08	+++	0	4, 8, 3
$(NH_4)_2SO_4 + CaCO_3$	Full arc	20	2 40	++	0	2, 3, 2
$(NH_4)_2SO_4 + CaCO_3 + ZnSO_4 + MnSO_4$	Full arc	20	2.40	+	0	6, 1, 1
$NaNO_3$	Full arc	7	23.25	Tr.	Tr.	1, 4, 4
Control	Uviol-filtered light	3	3.90	Tr	+	10, 9, 12
$(NH_4)_2SO_4 + CaCO_3 + ZnSO_4 + MnSO_4$	Uviol-filtered light	22	3.47	++	++	3, 0, 4
$NaNO_3$	Uviol-filtered light	4	27.70	++	+++	15, 15, 9

* Relative amounts of nitrite produced on inoculation of variously treated soils into Omeliansky solution. Incubation—10 days.

† 300 mgm. per 100 gm. of soil.

‡ 10 mgm. each per 100 gm. of soil.

the plates are reported in the table. On inoculating nitrifying solutions with the untreated and the exposed soils, the latter were found to give negative results, pointing to the destruction, by exposure to ultra-violet radiation, of the bacteria concerned in the process of nitrification. The only conclusion that could be drawn from this experiment is that a 2-hour period of irradiation had very little, if any, effect upon the oxidation of ammonia in soil or upon the reduction of nitrate and that it only resulted in an injury to some of the microbiological soil processes.

In the second experiment, only moist soil was used. It was divided into four 100-gm. lots receiving the following additions: nothing; $(NH_4)_2SO_4 + 300$ mgm. $CaCO_3$; $(NH_4)_2SO_4 + 1$ gm. $CaCO_3 + 10$ mgm. $ZnSO_4 + 10$ mgm. $MnSO_4$; $NaNO_3$. As in the first experiment, the nitrogen was added at the rate of 25 mgm. per 100 gm. of soil. The various lots of soil were divided before irradiation into three portions each, namely, (a) control, (b) irradiated with full arc for 6 hours, (c) irradiated with light filtered through a uviol filter for 6 hours. The soil samples were prepared on November 5, irradiated on November 10–11, and analyzed on November 13.

The results presented in table 2 show that irradiation for 6 hours, both by means of the full arc and by uviol-filtered light, brought about a decrease in the ammonia content of the soil to which the ammonium sulfate had been added. This decrease was not accompanied, however, by any increase in nitrate, except in the untreated soil. On the contrary, the nitrate content of the soil also decreased as a result of irradiation. Just what became of the

TABLE 3

Biological vs. photochemical oxidation of ammonium salt in soil

Milligrams nitrogen per 100 gm. of soil

ADDITION OF AMMONIUM SALT	EXPOSURE OF SOIL	AFTER TREATMENT			AFTER 2 WEEKS' INCUBATION		AFTER 5 WEEKS' INCUBATION[*]	
		$NH_4 - N$	$NO_2 - N$	$NO_3 - N$	$NH_3 - N$	$NO_3 - N$	$NH_4 - N$	$NO_3 - N$
−	−	3.8	Tr.	2.4	0	6.0	.	6.6
−	+	5.9	Tr.	2.2	6.0	2.8	.	4.8
+	−	27.3	0.042	2.5	11.6	22.0		22.0
+	+	24.9	0.063	2.2	26.0	3.2	16.0	10.9

* Inoculated with fresh soil after the previous analysis.

ammonia and the nitrate as a result of treatment is difficult to state, unless it was transformed into hyponitrous acid, as suggested by Corbet (2). The only conclusion that could be drawn from this experiment is that although the irradiation treatment had a slight effect upon the oxidation of ammonia, no nitrate was formed.

The irradiation with the full arc brought about a complete destruction of the nitrifying bacteria and a considerable reduction in the number of other soil organisms, as shown by the fungus count. Irradiation with filtered light did not destroy the nitrifying organisms and had only a slight effect upon other soil organisms. This confirms the results of many investigators (1, 4, 5) that the region effective in bactericidal action is below 3,000 Å. ZoBell and McEwen (7) failed to obtain, from a study of the vertical, diurnal, and seasonal distribution of bacteria in sea water, any evidence of lethal action of sunlight. In view of the fact that ordinary sunlight contains a very small quantity of the lethal rays, and even these are markedly reduced by passage through a very thin

layer of sea water and especially through soil, one would expect that the destructive effect of sunlight upon soil bacteria would be limited only to the very uppermost layer of the soil. This should have very little, if any, effect upon the soil microbiological population, as was shown previously (6).

In order to obtain further information concerning the specific effect of ultra-violet light upon the formation of nitrate in soil, another experiment was conducted. Four 100-gm. portions of soil were adjusted to optimum moisture; two received 25 mgm. of nitrogen as $(NH_4)_2SO_4$ and 300 mgm. $CaCO_3$, and the other two received no additional material. Two of the soil portions were exposed to the full arc for 5 hours, in 50-gm. lots, with constant stirring. The moisture was adjusted to optimum with distilled water. The soils were prepared on January 27, irradiated on January 30–31, and analyzed on February 2. After the first analysis, the soils were incubated in the original glass containers at 28°C. for 2 weeks, the moisture being kept optimum. The soils were again analyzed for nitrate and ammonia; they were then inoculated with a suspension of fresh soil and again incubated at 28°C.

The results presented in table 3 show that irradiation of soil did not bring about any oxidation of the ammonia to nitrate; actually it brought about some destruction of the nitrate. It resulted in a definite destruction of the nitrifying bacteria, as shown by the fact that, upon incubation, the transformation of the ammonia to nitrate was rapid in the unexposed soil, whereas the extent of oxidation in the exposed soils was very small. When these soils were inoculated with fresh soil, rapid oxidation of the ammonia began to take place in the exposed soils as well.

<div align="center">SUMMARY</div>

Results of three experiments on irradiation of soil lead to the conclusion that treatment of soil with ultra-violet light brings about the destruction of some of the soil microörganisms, including the nitrifying bacteria. Observations of numerous workers, who have shown that the maximum germicidal activity of light is obtained from radiations having a wave-length between 2,500 Å and 2,800 Å, were thus confirmed. No formation of nitrate in soil photochemically, however, could be demonstrated. In this respect, the soil behaves quite differently from sea water and liquid media.

<div align="center">REFERENCES</div>

(1) BACHEM, A., AND DUSHKIN, M. A. 1935 A study of bacterial sensitivity to ultra-violet radiation. *Biol. Bul.* 69: 109–125.

(2) CORBET, A. S. 1934 The formation of hyponitrous acid as an intermediate compound in the biological or photochemical oxidation of ammonia to nitrous acid. I. Chemical reactions. *Biochem. Jour.* 28: 1575–1582.

(3) DANIELS, F., AND HEIDT, L. J. 1932 Phytochemical technique. I. A simple capillary mercury vapor lamp. *Jour. Amer. Chem. Soc.* 54: 2381–2384.

(4) DUGGAR, B. M. 1936 Effects of radiation on bacteria. In *Biological Effects of Radiation* (Editor B. M. Duggar). McGraw-Hill Book Co., New York.

(5) HOLLAENDER, A., AND CLAUS, W. D. 1936 The bactericidal effect of ultra-violet radiation on *Escherichia coli* in liquid suspensions. *Jour. Gen. Physiol.* 19: 753–765.

(6) WAKSMAN, S. A., AND MADHOK, M. R. 1937 Influence of light and heat upon the processes of ammonia oxidation and nitrate reduction in soil. *Soil Sci.* 44: 361–375.

(7) ZOBELL, C. E., AND McEWEN, G. F. 1935 The lethal action of sunlight upon bacteria in sea water. *Biol. Bul.* 68: 93–106.

SOILS OF THE PHANEROPODZOLIC GROUP IN WESTERN OREGON

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All soils of the podzolic type of formation may be divided into two general groups, which may be designated as the phaneropodzolic soils and the kryptopodzolic soils. The phaneropodzolic group includes the soils characterized by a morphological profile giving a clear expression of the process of podzolization, whereas the group of kryptopodzolic soils is composed of the soils the morphological profile of which does not show the usual physical marks of podzolization and the podzolic character of which can be depicted only by chemical analysis. The development of a light ash-gray eluvial A_2 horizon may be considered the principal characteristic by which the soils of the phaneropodzolic group can be distinguished from the soils of the kryptopodzolic group. The difference in the B horizons of the two groups is less distinct and constant, although the B horizon of many of the phaneropodzolic soils is characterized by considerable compactness, whereas the same horizon of most of the kryptopodzolic soils displays no accelerated compaction.[1]

Several general groups of the phaneropodzolic soils are recognized, among which the following are the most prominent: normal podzols and podzolic soils; ground-water podzols and podzolic soils; gray forest soils; and meadow and glei-meadow podzols. All the true phaneropodzolic soils are the product of the woodland type of soil formation, which is characterized by a specific arrangement of the general biopedogenic cycle strikingly different from those of the grassland and the shrubland types of formation. Inclusion of the so-called meadow[2] and glei-meadow podzols in the phaneropodzolic group is therefore open to criticism. It is not at all certain whether these soils are genuine podzols or podzol-like soils. Indeed they have a definite morphological similarity to the genuine podzolic soils, although the mechanism of their formation may be different from that of the true podzols.

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[1] For a detailed description of the morphology, genesis, and chemistry of different podzolic soils, see Joffe (2) and numerous papers by the same author in different periodicals.

[2] The term "meadow" connotes the natural open areas, within the forested regions, occupied by dense herbaceous vegetation forming a compact sod. "Meadow soil" is an intrazonal soil of the grassland type within the forested regions. It is a dark-colored soil of the rather poorly drained open areas (half-bog, bog-meadow, Wiesenboden, etc.). The must conspicuous meadows, however, are in the valleys, especially on the lower terraces (flooded meadows).

In that part of the State of Oregon lying west of the Cascade Mountains are found the representatives of the ground-water podzols and their associates; the gray forest soils; and the glei-meadow podzols.

The geographic distribution, the physiographic and ecologic environment of formation, and the morphology of these soils were studied by the author in connection with a general investigation of the regional types of soil formation on the Pacific Coast, and a summary of the study is reported in this paper.

<div align="center">THE GROUND-WATER PODZOLS</div>

The ground-water podzols in western Oregon comprise a very small geographic unit. Moreover, all these soils are developed from one particular type of parent material the physical properties of which, apparently more than any other factor, were instrumental in conducting the local pedogenesis along the lines of typical podzolization. In the same physiographic region, other soils developed from different parent materials show hardly any marks of this process.

All areas of the ground-water podzols are stretched along the Pacific coast at the very foot of the Coast Range Mountains, which rise rather abruptly at some distance from the seashore to an altitude ranging from 1,000 to 2,000 feet. So far as is known at present, the northernmost area is in the southwestern corner of the State of Washington just north of the mouth of Columbia River. Several others are along the coast in Oregon, and one of the most prominent of these is north of Florence at the mouth of Siuslaw River.

These areas form the coastal flats which range in width, from the seashore to the base of the mountains, from less than a mile to about 5 miles. Some of them extend along the coast for more than 10 miles and are separated from one another by the stretches where the mountains drop abruptly to the sea. The elevation of the flats ranges from a few feet to little more than 50 feet above sea level. The topography of the flats ranges from gently undulating to moderately rolling. A number of small depressions without outlets are scattered among the smoothly rounded elevated tracts; the difference in altitudes between these is hardly greater than 20 to 30 feet anywhere. Many of the depressions are occupied by peaty bogs, and some are filled with water. The most prominent fresh-water lakes are situated at the foot of the mountains.

Most of the coastal flats are associated with the deltas of the westbound mountain streams and are fringed along the edges by chains of active sand dunes, some of which attain considerable height. In some places the dunes form a barrier blocking the outlet of smaller streams and creating a number of lagoons and lakelets, some of which are still open and others of which are filled with peat and form the sloughy bogs.

The flats are composed of loose and rather uniform sand, apparently of alluvial origin but thoroughly reworked and redeposited by the wind. The deep dune sand, fixed long ago by a well-established vegetation, may be considered, therefore, as the sole type of parent material from which the soils

of these areas are developed.   The depth of the ground-water table varies in different places from less than 2 to more than 10 feet below the surface of the ground.

The vegetation of the flats consists of an almost pure stand of pine (*Pinus contorta*) associated with the thickest evergreen underbrush composed of salal (*Gaultheria shallon*), rhododendron (*Rhododendron columbiana*), huckleberry (*Vaccinium ovatum*), and wax myrtle (*Myrica californica*), mixed with *Arctostaphylos columbiana*, *Arctostaphylos uva uvsi*, and several other relatively less common plants.

On the drier and more or less elevated areas very little, if any, herbaceous vegetation grows.   On the somewhat more open tracts the fern (*Pteridium aquilinum pubescens*) and several mosses and lichens (*Cladonia*) are not uncommon.   In wet boggy depressions the trees are scant and scrubby.   The shrubbery is principally Ledum (*Ledum columbianum*).   Here also are several sedges, several species of sphagnum, cranberry, sneak plants (*Sarracenia*), and other related plants of bog association.

The climate of the coastal lowland region is rather uniform throughout a considerable distance from north to south.   The average annual rainfall is about 70 or 72 inches.   The summer season, extending from June to September, is a decidedly dry period, the four summer months receiving less than 9 per cent of the annual total rainfall, or a little more than 6 inches.   The driest spell occurs in July and August, each of these months having about one-half an inch or less of rainfall.   The winter season, extending from November to March, inclusive, receives more than 70 per cent of the annual total rainfall, or more than 50 inches as an average.   During each of the three months November, December, and January, more than 10 inches of rain falls.

At different points of the coast, the mean annual temperature varies from 50°F. to about 52°, and it ranges from 40° to 45° in January to 58° to 61° in August.

The amount of rainfall at the higher altitudes on the slopes of the Coast Range rapidly increases to more than 100 inches a year.

Such is the natural habitat producing the typical ground-water podzols along the Pacific coast in Oregon.   The synthetic or average profile of this soil appears as follows:

A_0. A soft, puffy, and moldy mat of undecomposed vegetable residues, thoroughly interwoven by the roots of shrubbery.   It ranges in thickness from less than 1 inch to about 5 inches.   Usually it is thicker and more peaty on the flat, insufficiently drained depressions and not infrequently is absent on the dry, elevated or sloping areas.

A_1. Very dark gray, mellow to loose layer, ranging from 1 to about 8 inches in thickness and averaging about 3 to 4 inches.   The greater thickness is found on the flat or depressed areas having slow or poor drainage, whereas the entire horizon may be virtually absent on the elevated or sloping areas.

A_2. Very light ash-gray, in many places almost white, mellow to loose layer, ranging from less than 2 to more than 20 inches in thickness and averaging about 8 inches.   Typically it has a greater thickness and a lighter color (more nearly white) in depressed but relatively dry

B. Varies in color from spotted yellow-brown to very dark coffee-brown and in consistence from moderately compact to strongly indurated. Its thickness averages about 16 or 18 inches and ranges from about 10 inches to more than 2 feet. As a general rule this horizon has a brighter color and a stronger induration immediately beneath the A_2 horizon.

The typical ortstein occurs in low places having a relatively high water table (3 to 4 feet from the surface). In many places it forms a very dark coffee-brown or rusty brown firm, solid crust 1 foot thick. Its formation in most places is accompanied by a strong development of the A_2 horizon and a peaty A_0 horizon (pl. 1, fig. 1).

C. Very light gray, brownish gray, or olive-gray incoherent but, in many places, somewhat compacted sand, in some places, especially in low areas, slightly mottled with light iron stains.

Near and below the water table the parent material acquires a pronounced dull blue or greenish blue tint, is of a much darker shade, and gives off a strong odor of hydrogen sulfide.

The variations in characteristics of the individual profiles may be readlily correlated with the local topography. Plate 1, figure 2, shows the three typical and most common profiles of these soils.

THE WILLAMETTE VALLEY REGION

The gray forest soils and the glei-meadow podzols in western Oregon occur in the region lying east of the Coast Range Mountains and known as Willamette Valley. This is formed by a broad depression between the Coast Range and the Cascades extending southward from Columbia River. The elevations of this region range from 100 to about 500 feet above sea level. The central or axial part of the depression is occupied by the broad alluvial terraces of Willamette River; its topography ranges from virtually level to gently undulating. On both sides—east and west—the valley proper is bounded by the belts of smoothly rounded low foothills of the main mountain ridges.

The Coast Range Mountains separate Willamette Valley from the Pacific coast. The altitude of most of this ridge ranges between 1,000 and 2,000 feet above sea level, a number of peaks being between 2,000 and 3,000 feet high. The eastern (inland) slopes of the Coast Range merge with the floor of Willamette Valley with no abrupt change of elevation or of the general character of topography.

The Coast Range Mountains are formed mostly of sedimentary rocks. The soft pale yellowish brown Tertiary sandstones and associated shales are the commonest formations throughout the full extent of this ridge and the broad belt of its eastern foothills.

The coastal mountain ridge forms a barrier that divides the two different climatic provinces. Situated in the path of the oceanic moisture-bearing winds, it intercepts a large amount of rainfall on its western slopes, especially over the most elevated mountainous belt, and casts a dry climatic shadow over most of the intermountain depression. The difference of rainfall by the months in the coastal and the intermountain regions is shown on figure 1.

The average annual rainfall in the intermountain region is about 41 inches; that in the coastal lowland region, about 70 or 72 inches; and that in the high

mountainous belt, from 80 to more than 100 inches. The distribution of the total rainfall during the year is virtually the same on both sides of the mountains, only about 9 per cent of the rainfall occurring during the summer and 71 per cent during the winter. The average amounts for these periods are about 4 inches and 29 inches, respectively, as compared with 6 inches and about 50 inches for the same seasons on the coast.

The mean annual temperature in the Willamette Valley region is approximately the same as on the coast, 52.5°F. The annual extreme between the coldest and the warmest months, however, is considerably wider in the intermountain region than on the coast. It ranges from an average of about 40° in January to 66° in July and August.
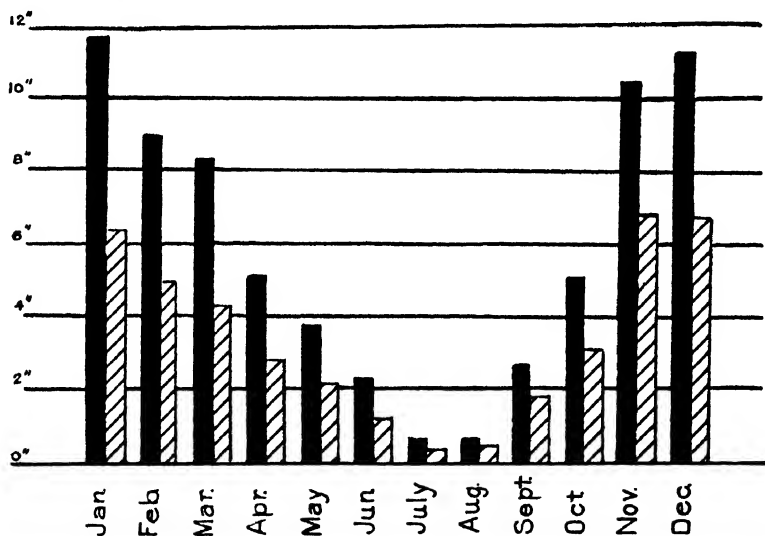
Fig. 1. Average Monthly Rainfall in the Coastal Region (black) and in the Intermountain Region (shaded) in Western Oregon

The dry and comparatively warm spell during the summer, together with a drastic general decrease of rainfall, apparently is responsible for a development in the low foothill region and in Willamette Valley of a general type of vegetation strikingly different from that on the seaward slopes. Whereas the Coast Range Mountains and the narrow coastal belt are entirely a dominion of luxuriant evergreen forests composed mostly of Douglas fir, hemlock, and cedar, with a dense underbrush of evergreens and ferns, the oak (*Quercus garryana*) is the most conspicuous tree in the low foothills east of the Coast Range. Not infrequently almost pure stands of oak with only scant deciduous underbrush and herbaceous plants occupy the extensive gently rolling or hilly areas. In many other places the oak trees are scattered sparsely over the open tracts covered by grasses.

Another conspicuous feature of the landscape in the foothill region is the

open southern slopes of many hills, occupied by a rather thick grass vegetation. The northern slopes of the same hills are covered by thriving oak groves, with groups of fir and other coniferous trees occupying the same slopes at some distance from the crests. Such an asymmetric distribution of vegetation is particularly prominent throughout the foothill region extending west of Willamette River from the town of Dallas to the southernmost part of the valley (pl. 2, fig. 1).

Because of the almost continuous cultivation of the land since the time of its settlement, it is rather difficult to re-establish with certainty the original character of the vegetation of the broad alluvial terraces of Willamette Valley. It is generally known, however, that prior to the settlement of the valley, the wide, entirely open, or sparsely timbered grass-and-sedge meadows occupied much of the broad terraces and were intermingled with the dense mixed valley forests, most of which were along the banks of streams.

THE GRAY FOREST AND RELATED SOILS

The gray forest and related soils occur in many places throughout the lower belt of the eastern foothills of the Coast Range Mountains. The development of these soils is definitely associated with the oak forests. By far the greatest area is developed from a residual parent material formed by the weathering *in situ* of the Tertiary sandstones and associated shales. In general these parent materials are not very deep: the soft decayed bedrocks in most areas lie at a depth ranging from less than 2 feet to about 7 feet below the surface. A smaller area of the gray forest soils is developed from products of weathering of igneous rocks, mostly basalts, or from transported and redeposited materials.

The topography of the entire region is characterized by broad, well-rounded, gently sloping, low hills gradually merging with the adjacent plains of the valley floor. The surface drainage is good.

Most of these soils were recognized by the older soil surveys as members of the Carlton series. It has been roughly estimated that the soils of this series alone occupy an area comprising about 100,000 acres. The total area of the gray forest soils here, however, is undoubtedly much larger.

The synthetic or average profile of the virgin gray forest soil (Carlton silty clay loam) appears as follows:

A_0. A light, puffy, and moldy surface covering, ranging from 1 to 3 inches in thickness and composed of dry leaves, acorns, pieces of bark, rotten wood, and other residues, interwoven by the thin roots of shrubs.

A_1. Dark-gray, in many places almost black at the surface, ranging in thickness from 2 to 6 inches. In most places it has a well-developed lumpy or crumbly structure.

A_2. Light or very light gray, in some places tinted slightly yellow, averaging about 16 inches in thickness and ranging from 8 inches to more than 2 feet. The upper part of the horizon is considerably darker colored than the lower part.

The lighter colored lower subhorizon is characterized by the occurrence in many places of white "podzolic flour"—the finest quartz powder sprinkled in cracks, pores, and other voids

throughout the entire subhorizon.   In many areas the podzolic flour is present in considerable quantity, filling the cracks and voids among the structural aggregates or forming rather heavy coatings on their surfaces.

The entire A_2 horizon has a moderately developed to well-developed medium lumpy (nutlike) structure.   The aggregates are irregular and rather sharply angular in shape, have a somewhat tough but crumbly consistence, readily separated from one another, and range in size from less than one-fourth inch to about one-half inch.   For the most part they are somewhat larger in the lower subhorizon, although in a few places the reverse condition can be observed.

B. Yellowish brown, reddish brown, dull brown, or brownish gray, averaging about 12 inches in thickness, with variations from 8 to about 20 inches.   It has a compact and tough consistence; its texture is considerably heavier than that of the A_2 horizon; it breaks into well-developed medium to coarse lumpy (blocky) aggregates.   The structure of this horizon is typically best developed at the top of the horizon.

Very few grass rootlets penetrate the B horizon, whereas by far the greatest number of the tree roots (oak) are distributed in this horizon (pl. 2, fig. 2).

The lower boundary of this horizon is rather indistinct and irregular where it grades into the unmodified but thoroughly weathered parent material.   In many places, however, the B horizon rests directly on the decayed, strongly fractured sedimentary bedrocks (sandstone and shale), which are brightly and non-uniformly colored pale yellow, orange, reddish brown, and brown.   The depth of the ground-water table ranges from 25 to about 40 feet.

A typical feature of the Carlton soils is a considerable, and often even strong, reworking of their material by earthworms, the burrows of which are especially abundant in the A_2 horizon.   Powers and Bollen (4) estimate the number of earthworms in some other soil types of the same region as ranging from 500,000 to 1,500,000 an acre, which may give an average earthworm population ranging from 5 to almost 20 worms per cubic foot of soil.   The A_1 horizon and the top section of the A_2 horizon in many places are perforated also by the burrows of small rodents, especially field mice.

The soils of the Carlton series geologically are closely related to the soils of the Melbourne series,[3] with which they are connected through a series of intermediate or transitional phases in the foot-hills region.   It should be noted that the gray Carlton soils are associated directly with the oak forests, whereas the brown or reddish brown Melbourne soils are the products of the evergreen, and especially of coniferous, forests.   In the transitional belt, however, such a correlation of the soil and flora is not so close: the changes of the soil characteristics, although distinct, are in many areas more gradual than those of the types of vegetation.   Probably this is due to the relatively faster rate of re-

[3] The Melbourne series as it stands now comprises a group of soils larger than one series. A more detailed investigation undoubtedly will establish several independent series within this group.   A habitat of the true Melbourne soil is characterized by a residual parent material formed by weathering *in situ* of Tertiary sandstones and shales, a dense evergreen forest vegetation (Douglas fir, hemlock, and cedar, together with a thick underbrush and ferns), a very humid and mild marine climate (60 to 100 inches of annual rainfall), and a mountainous to hilly topography.   These soils occur in the Coast Range Mountains in southern Washington, in Oregon, and in northern California.   The brilliantly colored "red" soils of this group comprise an independent (Sites) series.

placement of one type of vegetation by the other than that of the accompanying evolution of one soil type from the other.

The general morphological characteristics of the Carlton soil, especially the structure of the A and B horizons, an occurrence in many places of podzolic flour in the A_2 horizon, and an accelerated compactness of the B horizon are the typical features of the gray forest soils.

Many soil students regard the gray forest soils as secondary podzols, that is, as soils remodeled by the podzolic process from originally different soils. The secondary podzols thus are contrasted with the original or primary podzols, which presumably are developed directly from the virgin parent materials that had never been affected by any other pedogenic process.

It is assumed that formation of the gray forest soils is due to degradation of the chernozems (1) caused by an invasion of the open grassland region, like the steppe, by forest vegetation. Such an invasion has been made possible, according to the theory of degradation, by changes in the climate, and naturally it has been accompanied by a fundamental rearrangement of the general biopedogenic cycle.

The probability of degradation is supported by a gradual change of the physical and chemical properties of the northern chernozems through the transitional geographic belt, between the regions occupied by the unmodified chernozems and the stabilized gray forest soils, which presumably has been invaded rather recently by the advancing forest. Further evidence has been furnished by a laboratory experiment with an artificial leaching of the chernozem soil covered by a layer of oak leaves that changed this soil into a soil similar to the natural gray forest earth.

The advance of forest into the steppe, however, has not been proved with certainty for this region. Apparently also it has not been fully appreciated that the progressive changes of the soil, accompanying its regeneration from one type into another type, produce a series of other morphological profiles, successively replacing one another, that are similar to the profiles of the stationary intermediate phases linking the two extreme types if the latter occur simultaneously in two adjacent regions. It is possible that in many instances the gray forest soils are formed by the climax degradation of chernozems. In many other instances, however, similar soils are developed directly from the original parent material.

In all probability the gray forest soils of western Oregon are the original soils. There is no certain evidence that this part of Oregon was ever occupied by the subarid steppe which was invaded subsequently by the forest vegetation, as should have been the case if the Carlton soils were the climax of degradation.

It is true, however, that the isolated areas of the open southern hill slopes represent an environment very similar to, if not identical with, that of the steppe, and that the dark-colored, almost black, soils resembling morphologically those of the dry prairie type, occur on many of these sun-baked slopes.

Nevertheless a close geographic association of the two physiographic climaxes, that of the open southern slopes and that of the forested northern slopes, offers no reason for an assumption of some evolutional relationship between them. It appears almost certain that the grassland climax of the sun-baked areas is so well established, either geographically or ecologically, within the fundamentally stabilized boundaries that no natural changes of these boundaries were or are possible since the stabilization of this general geomorphologic landscape. These rather abrupt boundaries strictly following the crests of hills, are fixed by static physical conditions rather than by the dynamic forces of competing vegetation (grass and oak). It is hardly possible, therefore, that the habitat of grassland ever extended beyond the limits of its present distribution, especially toward the northern slopes, or that some of this territory is, or may have been, a subject of invasion and conquest by the forest.

THE GLEI-MEADOW[4] PODZOLS AND RELATED SOILS

The glei-meadow podzols occupy a number of rather extensive tracts on thr broad, flat, and generally insufficiently drained terraces of Willamette Rive-Valley. Most of them are developed from heavy and compact alluvium come posed of clay mixed with various proportions of silt. As has been mentioned, no precise correlation of any type profile of these soils with any particular plant association is possible at present because of a thorough change of the original habitat by cultivation. It is known, however, that the areas of these soils were mostly occupied by the grass-and-sedge meadows. These soils are known locally as "white land" and were recognized by the older investigations as members of the Dayton series. Closely related to these, but darker, are the soils of the so-called "half-white land," comprising the Amity series.

The synthetic type profile of the glei-meadow podzol in Willamette Valley (Dayton silty clay loam) appears as follows (pl. 3, fig. 1):

A_1. Gray or dark gray, 2 or 3 inches thick, so thoroughly interwoven by the abundant grass rootlets that a compact sod is formed. The soil material is more or less granulated.

A_2. Light gray or very light gray, averaging about 12 inches in thickness and ranging from

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[4] Glei is a horizon of the soil profile (or the material of such a horizon), characterized by restricted aeration, caused mostly by saturation with water, and by a considerable reduction of the higher oxides, especially the oxides of iron. Because of a reduction of the ferric compounds to the ferrous compounds, the soil acquires a dull olive-gray color with a pronounced blue or green tint and usually mottled with rusty stains. It is assumed that a reduction process may be caused by the anaerobic microorganisms which separate the oxygen of the higher oxides and utilize it for their needs, such as synthesis of protoplasm. The glei horizons are a typical feature of all permanently or seasonally poorly drained soils, and generally they develop at a distance from the surface sufficient for a stabilization of the anaerobic biopedogenic activity. The rusty mottling is not an essential characteristic of the glei, but nevertheless it is very common. It is caused by a local oxidation of the ferrous compounds during the dry seasons when the cracks, pores, root channels, and other voids are open for the free circulation of air, or when oxygen is introduced into the glei by the living roots.

6 inches to more than 2 feet. It is thoroughly penetrated by the finest white podzolic flour which gives to it its exceedingly light color. This podzolic flour appears from the very top of the A_2 horizon as a moldlike sprinkling in the cracks, pores, and other voids, and it gradually increases in quantity in the deeper parts, reaching a maximum at the base of the horizon.

The entire A_2 horizon has a rather compact consistency and a very fine to medium lumpy structure or in fewer areas, a platy structure. On drying, it "bakes" into a solid and rather firm very light gray, almost white mass. Persistent rubbing reduces it to a soft floury powder.

The occurrence, in great numbers, of small well-rounded dark brown or black ferric concretions (ortstein) is a typical feature of this horizon. The concretions range from those hardly visible to the naked eye to those about $\frac{1}{16}$ of an inch in diameter and generally are firm enough to resist crushing, even by strong pressure. They appear in the soil from the top of the A_2 horizon and are especially numerous in the A_2 lower subhorizon.

B. Very dark drab gray, dull gray, or dark olive-gray, ranging from 8 to about 20 inches in thickness and averaging about 15 inches. It consists of heavy, very compact clay that has a sticky and watertight consistence when the soil is wet and breaks into coarse firm prismatic clods on drying. The prisms range from less than 2 to about 4 inches in diameter and extend through the entire thickness of the horizon; in other words, their vertical axes are two or three times longer than the horizontal axes. The prisms separate from one another rather easily, being divided by coarse vertical cracks. These cracks extend through the whole thickness of the B horizon but do not extend into the A_1 and A_2 horizons and do not penetrate deeply beyond the base of the B horizon (pl. 3, fig. 2). The tops of the prisms are rather irregular. The podzolic flour so abundant in the A_2 horizon disappears rather abruptly at the top of the B horizon; however, a close examination of the boundary between these two horizons shows that the development of the bleached A_2 horizon proceeds through a fundamental podzolic digestion of the underlying horizon from the tops of its prisms. A few iron concretions of a diameter not greater than $\frac{1}{18}$ of an inch may occur here and there in the B horizon. The lower boundary of the B horizon is rather indistinct: the cracks diminish in number and size and extend in a less strictly vertical direction, and the color of the soil gradually merges into that of the parent material.

C. The parent material, olive-gray of a much lighter shade than that of the B horizon and with an increasingly strong yellow or light brown tint and a slight mottling with rusty iron stains in the lower strata. The content of silt and in many places of fine sand gradually increases with depth, thus reducing the compactness and toughness of the material. The normal depth of the ground-water table ranges from less than 10 to about 20 feet below the surface.

The watertightness of the B horizon in wet soil causes a waterlogging of the upper horizons during the wet seasons—winter and spring. On the average, such a condition exists during a continuous period of not less than 6 or 7 months annually. The lack of either surface or internal drainage maintains a seasonal anaerobic habitat in the soil and naturally stimulates a very effective glei formation. It is not definitely known whether the same condition can be held responsible for a formation of the iron concretions in the A_2 horizon. A prevailing opinion among soil scientists is that these concretions represent a variety of ortstein (1) and that their formation is caused by a hydrologic regime of the soil. This does not explain, however, a dissemination of the ortstein into a great number of isolated small grains or a concentration of the iron compounds at and around certain points only. That these concretions may be formed by colonies of certain iron-fixing microorganisms is a possibility.

The other meadow-podzolic soils related to the Dayton type differ from it in several respects. The most significant changes occur in the A_1 and B horizons. The A_1 horizon, which in these soils embraces the upper part of the A_2 horizon, acquires a dark gray or very dark gray color and a greater thickness, ranging from 1 to about 2 feet, caused by a considerable accumulation of humus. The A_2 horizon—equivalent of the lower part of the A_2 horizon of the Dayton soil—in some areas is reduced to a rather slight sprinkling of a podzolic flour in the lowest part of the A_1 horizon and on top of the B horizon. In many places the deeper part of the A horizon is bleached instead of being sprinkled with podzolic flour. Such a bleaching may appear in the form of diffused light spots intermingled with the darker areas. In many places the tough and compact B horizon is developed less conspicuously and may even be absent.

It was pointed out in the first part of this paper that an inclusion of the so-called meadow podzols with the other podzols and even their names are open to criticism.

An objection to the assumption of the podzolic character of the bleached meadow soils is based on the fact that these soils are formed in a grassland habitat, whereas development of the genuine podzols is definitely associated with a biopedogenic cycle maintained by forest vegetation.

A brief comparison of the two types of the biopedogenic cycles may be set forth as follows:

The herbaceous vegetation turns over to the soil every year, virtually *in toto*, the bulk of living matter built during a vegetative period, whereas the trees deposit their residues in two installments. The annual deposits, consisting of dead leaves, needles, cones, fruits, pieces of bark, and dry branches, are combined with the residues of the forest grasses, mosses, lichens, and ferns. The main bulk of woody material is deposited at longer intervals; this slows down the tempo of migration through the biosphere of a considerable part of the elements circulating through the soil and the living matter.

The main bulk of residues of forest vegetation is deposited on the soil *surface*. The residues of roots left *within* the soil comprise a small part of the total amount, and this is distributed throughout a considerable thickness of the soil horizons. The ratio between the amounts of organic residues deposited on the surface and within the soil by the grass vegetation is more or less the reverse of this. Besides, the bulk of grass roots is concentrated in a relatively thin A horizon.

The decomposition of organic residues in the habitat of a grassland proceeds rather slowly, extending over a number of years, which leads to the formation of a considerable amount of humus. Mineralization of the residues in a woodland habitat proceeds faster than that in the grassland, and it is not accompanied by the formation of true humus.

It appears, therefore, that a general biopedogenic cycle of the woodland type proceeds rather fast through its soil sector and slows down in passing through

the biosphere, whereas the cycle of the grassland type proceeds more or less rapidly through the biosphere and slows down in passing through the soil.

Development of the specific profiles by the woodland or grassland soils depends to considerable extent on the distribution of roots in different horizons. Most of the grass roots are concentrated in the A horizon near the surface of the soil. Consequently in this soil the mineral plant nutrients are liberated from the organic residues and are consumed in virtually the same horizon.

The greatest part of the roots of trees is distributed in the B horizon, at some distance from the surface, whereas most of the residues decompose on the surface. The migrating substances, therefore, must percolate through the A horizon before they reach the roots. Such percolation proceeds in an acid solution. Passing through the A horizon, these solutions affect the skeletal material of the soil, hydrolyze, decompose, dissolve, and carry down certain compounds of the soil skeleton in addition to the essential plant nutrients. The roots intercept most of the solvent and a selected part of the dissolved compounds, which naturally changes the concentration of the nonconsumed compounds brought down from the A horizon and causes their precipitation and accumulation.

To a considerable extent the distribution of roots in different horizons itself stimulates and directs a movement of soil solutions upward and downward because of sucking and transpiration of moisture by the roots.

Development of the bleached eluvial A_2 horizon and of the illuvial B horizon of the true podzols is caused by a predominantly downward percolation of the soil solution, without which apparently no genuine podzolic profile can evolve. The bleached meadow soils are developed under a grass vegetation. Their morphological profile, however, is similar in several respects to that of the strongly podzolized soil and, to the contrary, has very little in common with the profile of a typical grassland soil.

Such a discrepancy between the habitat and the morphology of the profile suggests that a podzol-like morphology may evolve in more than one way. The presence of a watertight B horizon, a waterlogged condition of the upper horizons of the soil for more than half of the year, and a strong development of the glei lead to the conclusion that a steady downward movement of the solutions and the leaching are hardly possible in these soils and that some factors other than a true podzolization are responsible for a development of these soils. Whether it is a hydrolysis or something else is still not precisely known.

The glei-meadow podzols, like the other meadow soils, belong to the group of intrazonal[5] soils. It is interesting to note that the morphology of the glei-

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[5] Three general groups of soil—zonal, azonal, and intrazonal—are recognized on the basis of their geography. The *zonal* soils are those whose genesis is a product of the environment controlled by the laws of geographic zonality (climate, ecology, biologic pressure, etc.). The *azonal* soils are those whose development is independent of the forces of geographic zonality; for example, the soils of the steep mountain slopes, the soils of the alluvial terraces subject to periodic overflow, or the soils of the regions of active sand dunes. The *intrazonal* soils are those whose development is controlled by some local factor. These soils occur in relatively small areas scattered throughout the territory occupied by the zonal soil. The commonest example of an intrazonal soil is the hydromorphic soils of depressions.

meadow podzols is strikingly similar to that of the so-called "solodi"—an intrazonal soil found in the grassland belt (prairie and steppe) where it similarly occupies the depressed and poorly drained areas.

GENERAL DISCUSSION

The three different soils having a distinct morphology of the phaneropodzolic type which occur in western Oregon were examined by the author. Of these three soil types the ground-water podzols and the glei-meadow podzols are rather local formations, whereas the gray forest soils of the eastern foothills of the Coast Range Mountains appear as the normal regional soil of this geomorphologic landscape. In western Oregon these soils form an island widely separated from the main zone of the podzolic soils. The occurrence of this island is due to a Cascadian inversion of the great soil zones on the Pacific coast and its reinversion by the Coast Range Mountains.

The general character and significance of the Cascadian inversion has been described in another paper (3) as follows: The Cascades form a barrier which arrests an inland spread of the oceanic moisture-bearing winds and separates the humid coastal belt from the arid region extending east of the mountains. The humid coastal belt is a region of exceedingly high biologic pressure, whereas the area east of the mountains is a region of very low biologic pressure (semi-desert). This distribution of the climatic and biotic forces causes a corresponding distribution of the major soil groups. The westward extension of the chernozem zone is abbreviated in the eastern part of the State of Washington by a northward protrusion of the semidesert zone that occupied the arid region east of the Cascades. This in turn is replaced by the belt of bright-colored soils of the humid zone.

This general scheme is complicated in Oregon. The typical chernozems occupy a relatively small area in the northeastern part of the state. An extension of this area eastward and southward is limited by the Rockies and the Blue Mountains. The Blue Mountains extend in a northeast-southwest direction and cut across the northward protrusion of the semidesert. The elevation of these mountains ranges from 4,000 to more than 6,000 feet above sea level, which causes an increase in rainfall and a thriving growth of forest vegetation. A Blue Mountain vertical zone separates almost completely the Columbia Basin island of the semidesert from the semideserts of southeastern Oregon, southern Idaho, and areas farther south.

West of the Cascades the general scheme of the inversion is modified especially by the Coast Range Mountains that form the first moisture-arresting barrier between the seacoast and the Cascades. The Coast Range Mountains perform a rôle similar to that of the Cascades: they arrest a part of the moisture carried by the oceanic winds and cast a dry climatic shadow over a large part of the intermountain depression. Consequently the humid coastal zone is split here into the two meridional belts, one lying west and throughout the Coast Range, and the other west and through the Cascades, separated from each other by a relatively dry intermountain belt.

The dry climatic shadow caused by the Coastal Range is less dense than that caused by the Cascades. The normal annual rainfall east of the Cascades ranges from 6 to about 15 inches, whereas that east of the coastal mountains is about 40 inches. Naturally, the reinversion caused by the Coast Range therefore does not create a habitat of the semidesert with its climate, vegetation, and soils, but its climatic and biotic conditions are sufficient for a fair development of the gray forest soils.

A reinversion of the humid coastal zone is already in evidence in southwestern Washington where it is caused by the Olympic Mountains, although no gray forest soils are known throughout the region of the Olympic reinversion. Instead several areas are occupied by the black prairie soils (xerophytic meadow). Generally speaking, development of prairie soils requires a climatic condition somewhat drier than that under which the gray forest soils are formed. The amount of rainfall is virtually the same in both regions, that east of the Olympic and that of the Coast Range Mountains. A sufficient difference in humidity as a factor of soil formation may be caused, however, by the differences in parent materials. The prairie soils of western Washington are developed from a very porous and gravelly fluvioglacial deposit having an exceedingly low moisture-holding capacity. In connection with this it should be noted that similar dark-colored soils (Sifton series), developed from an identical parent material, are found in some localities east of the Coast Range in Oregon.

REFERENCES

(1) GLINKA, K. D. 1935 Pochvovedenie (Pedology), ed. 4 [Russian]. Moskow-Leningrad.
(2) JOFFE, J. S. 1936 Pedology. Rutgers University Press, New Brunswick, N. J.
(3) NIKIFOROFF, C. C. 1937 The inversion of the great soil zones in western Washington. Geogr. Rev. 27: 200–214.
(4) POWERS, W. L., AND BOLLEN, W. B. 1935 The chemical and biological nature of certain forest soils. Soil Sci. 40: 321–329.
(5) TORGERSON, E. F., AND POWERS, W. L. 1928 Meaning and use of Willamette soil survey. Oreg. State Agr. Exp. Sta. Cir. 90. [See also soil survey reports of the Bureau of Chemistry and Soils, U. S. Department of Agriculture.]

PLATE 1

FIG. 1. A strong ground-water podzol with ortstein on the sandy coastal flats in Oregon.

FIG. 2. Typical profiles of the ground-water podzols in western Oregon: (a) strong podzol in a low-lying area having a high water table; (b) well-developed podzol on the well-drained level or gently undulating areas; (c) weak podzol on the dry sloping or rolling areas.

FIG. 1

a *b* *c*

FIG. 2

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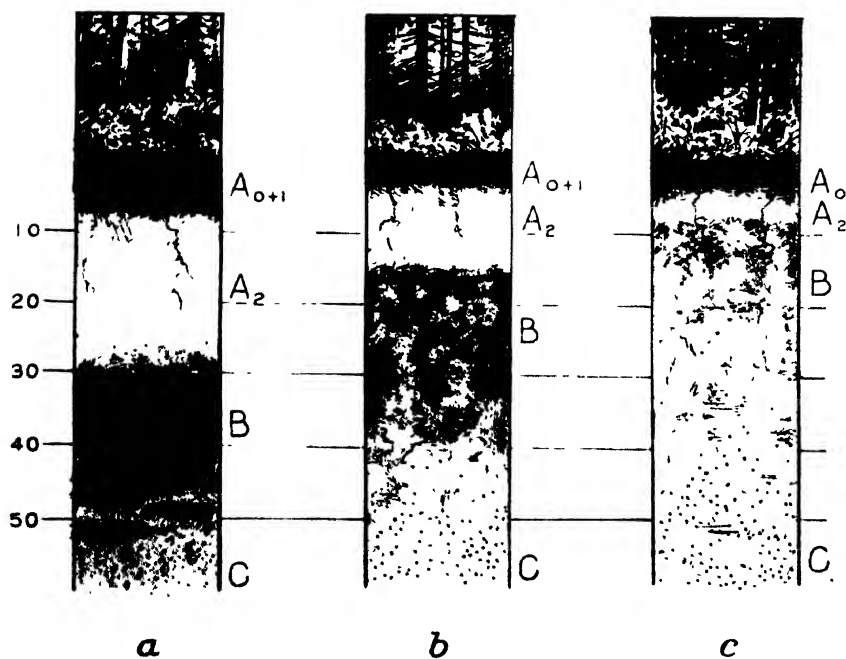
PLATE 2

FIG 1 Asymmetrical distribution of vegetation in the eastern foothills of the Coast Range Mountains
FIG. 2 Distribution of tree roots in the B horizon of the forest soil

FIG. 1

FIG. 2

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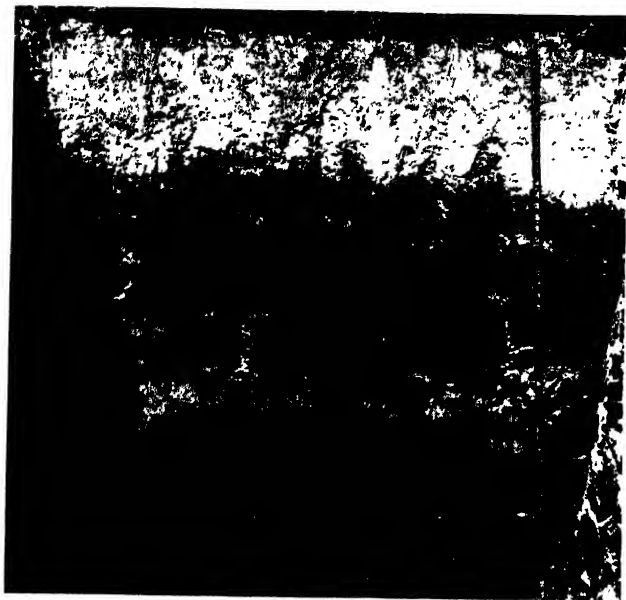
FIG. 1

FIG. 2

PORE SPACE DETERMINATION AS A FIELD METHOD

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Soil structure can be determined by several methods, each of which takes into account certain physical properties of the soil. Structure may be characterized by the form and magnitude of the soil crumbs or of the soil pores. The stability of the soil crumbs or of the entire soil is also important. The magnitude of the soil crumbs is found in the aggregate by determining the pore space (1, 2, 14, 21), and in more detail by the sieve tests of Tiulin (20), Cole and Edlefsen (4), and Keen (8) or by permeability measurements, as mentioned by Russell (15). The kind of soil structure is determined by direct examination (10, 11, 16, 17, 18, 19). The stability, or cohesion, can be determined by the method of Tiulin (20) or by the friability test, as designed by Christensen (3) and afterward used by Webb, Jennings, and Peterson (22). All these methods are laboratory methods and can be carried out in the field only with extreme difficulty. Furthermore, they are impractical for large-scale determinations.

For field work several instruments have been designed for measuring the resistance of the soil to the insertion of a pin. These instruments, which range from simple tools to the most intricate apparatus (5, 6, 7, 9, 13), would be of considerable use in solving agricultural problems, because a large quantity of determinations can be made rapidly, were it not for the fact that the method itself is of questionable value. According to our experience, the determinations are extremely complicated and are influenced to a varying degree not only by pore space and cohesion, but also by the friction of the soil along the pin, by the elasticity of the soil, by the direction in which the pin is inserted into the soil (vertical or horizontal), by the depth to which the pin is inserted, and by the presence of holes or cracks in the immediate vicinity of the pin (within a circle of about 20 cm.). Consequently, one never knows whether one has determined pore space, elasticity, or some other property.

In work on the lodging of rye we devised a procedure for determining pore space on a large scale. The data obtained by this method were of more absolute value than those obtained by the instruments previously mentioned.

Field work demands of an instrument special qualities beyond the require-
ments of scientific exactness. The instrument must meet the following
requirements:

It must be easily handled and allow a quick determination.
It must be sufficiently exact and should give as accurate results as possible.
It must be so designed that the absence of level tables will not interfere with the readings.
It must be able to withstand dirt and handling with dirty hands.
It must be strongly constructed: any damage due to loss or breakage must be repairable
immediately.

PRINCIPLE OF THE NEW INSTRUMENT

The instrument designed, following the example of Torstensson and Ericsson
(21), is based on the principle that if a volume of air is compressed, the
volume and the pressure are related, according to Boyle's law. If the volume
is not known, it can be calculated from two pressure readings and the variation
in volume that accompanies the variation in pressure. In using in the field
one of the instruments previously mentioned, difficulties arise mainly from the
manometer. The open construction requires rubber tubing, a level position,
and mercury, all of which are inconveniences. Several advantages would
arise from the use of a glass tube, closed at the top, as a manometer. As a
matter of fact, the part of the apparatus in which the soil sample is placed—
"the soil chamber," as we shall call it—may be considered as a manometer
closed at the top. This means that the instrument consists of two manometers
of the same type, but with different heights. Placing a soil sample in the soil
chamber of the instrument and raising the pressure will produce the same
effect as shortening the volume tube, for a decrease in volume by constant
diameter of the tube, means a shorter apparent length. This apparent
length can be calculated, for it will be clear that if in the manometer part one
half of the original volume of air is occupied by water, one half of the volume
of air in the soil chamber also will be replaced. If we can measure that volume,
the whole volume of the soil chamber will be known. The difference between
the blank and this calculated volume is the volume of the soil sample, that is,
the volume of the soil together with the water contained in it.

A linear relation exists between the readings of the water level in the two
tubes. This facilitates determining the volume of a soil sample, for two
arbitrary readings will allow calculation of the volume. Let us designate the
length of the manometer tube l and the apparent length of the volume tube p
(fig. 1). If, further, we assume that two determinations are made, with pres-
sure readings on the manometer equal to x_1 and x_2, and the corresponding read-
ings of the volume tube equal to y_1 and y_2, then the following relation holds:

$$\frac{l - x_2}{x_2 - x_1} = \frac{p - y_2}{y_2 - y_1}$$

or

$$p = y_2 + \frac{(l - x_2)(y_2 - y_1)}{(x_2 - x_1)}$$

The difference between the length m, which is calculated from a blank trial, and the length p, found when a soil sample is placed in the volume tube, multiplied by the area of the transverse section of the volume tube gives the volume of the soil sample, in cubic centimeters.
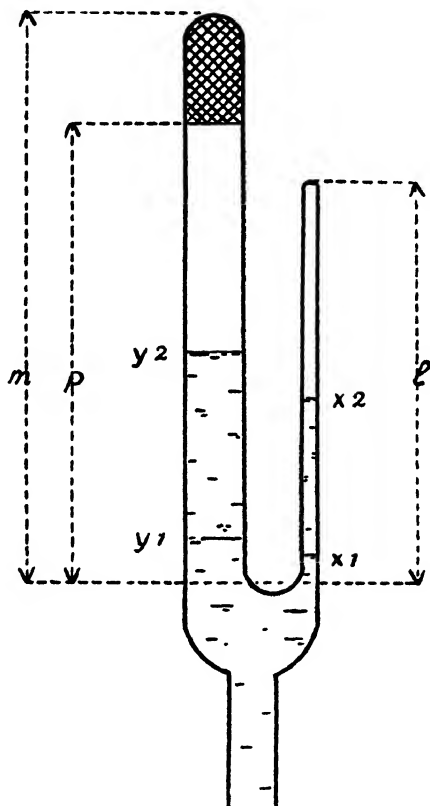
FIG. 1. DIAGRAM SHOWING THE PRINCIPLE OF THE NEW INSTRUMENT

CONSTRUCTION AND USE OF THE APPARATUS

To ensure strong construction the whole apparatus was mounted in a 7.5-cm. copper tube, as shown in figure 2 and plate 1.

Chamber a is filled with water, which is compressed with a bicycle pump through valve b. This forces the water into the manometer tube c, which is fixed at the bottom of the pycnometer to prevent air bubbles from entering the manometer. The water from chamber a enters the volume tube through
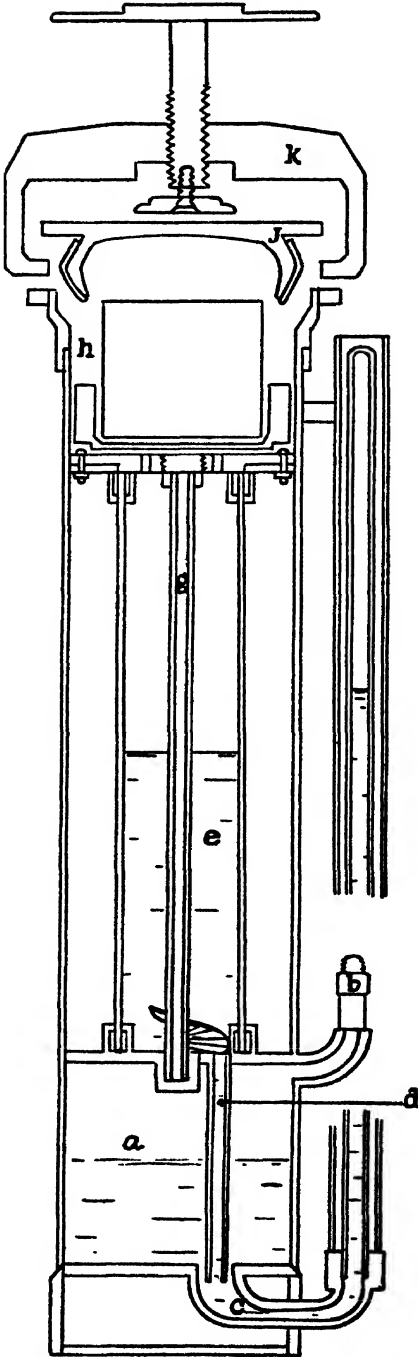
FIG. 2. DIAGRAM SHOWING THE CONSTRUCTION OF THE APPARATUS

tube *d*, which is small and does not reduce greatly the volume of chamber *h*. This small tube ends in the opening of the manometer so that air bubbles are washed away by the water which streams into the volume tube and so that all the water from chamber *a* can be forced into the volume tube. Over the end of the small tube *d* a piece of copper sheeting is mounted in a slanting position which bends the water stream and causes tube *e* to fill quietly.

The volume tube *e* is made of glass and is fixed to chamber *a* and chamber *h* by means of copper fittings sealed with a mixture of litharge (PbO) and glycerine.

The displacement of the air by water is measured on the scale *g*, placed in the center of the glass tube. This construction precludes to a large extent errors arising from deviations from the vertical position. The soil sample is placed in chamber *h*, with or without the ring in which the sample was taken. This chamber can be closed by means of the cover *j*, fitting on top of a copper cylinder with a slanting rubber packing ring. The slanting surface keeps soil particles from sticking to it, thus ensuring an air-tight connection. A metal ring with a screw *k* keeps the cover in place.

The volume readings are made on the central scale at the lower side of the water surface. The pressure is read from a scale along the manometer tube. Around the glass tube a copper tube is mounted as a guard against impacts. The water level can be seen through slits in the copper mantle. The scale is placed on the edge of the slit, and a sliding index inside the copper mantle allows quick, accurate readings.

The apparatus is constructed for a pressure of 2 atmospheres. In our opinion this pressure is of great value for accurate work. The greatest precision is reached when the greatest variation in pressure is accompanied by the greatest variation in volume, which can be read from the instrument. In order to meet this requirement, an ebonite cup containing the soil sample is placed in chamber *h*. This cup has walls of such thickness and occupies such a volume that with an average sample the full scale of the volume tube and of the manometer tube can be used.

The samples are taken with a chromium plated steel ring 5 cm. high and 5 cm. in diameter and with walls 1 mm. thick. One edge of the ring is slightly sharpened. This sharp edge, together with the smooth chromium surface, allows the ring to be pressed by hand into any soil that is not unusually compact. (Using a hammer disturbs the structure of many soils.) The soil is then cut away with a knife along the lower and upper edges of the ring while the ring is still in the soil.

ACCURACY OF THE DETERMINATION

The instrument is characterized by three constants; namely, the total volume of the soil chamber, the length of the manometer tube, and the volume of the central cylinder to a height of 1 cm. The volume of the soil chamber is calculated from a blank trial. The length of the manometer tube

can be measured directly along the manometer scale.   The volume of 1 cm. of the central cylinder is calculated from the difference in height of the water level before and after pipetting 100 cc. of water into the central cylinder.

To get some idea of the accuracy of the method and of the errors arising from the porous nature of soil, the volume of three steel cylinders of known volume and the volume of a soil sample were determined.

The soil sample was taken from a clay soil that had not been plowed for 3 years.   Every year sugar beets were grown on this experimental field.   The soil is characterized by the following values:

pH		7 75
Sorbed bases	m.e.	38 7
Total base exchange capacity[2]	m.e.	41 0
Saturation value	per cent	94 4
CaCO$_3$	per cent	6 44
Mechanical analysis >100μ	per cent	3 8
100–75μ	per cent	0 6
75–50μ	per cent	8 3
50–35μ	per cent	11 3
35–20μ	per cent	17 0
<20μ	per cent	50 2
Humic material	per cent	2 3

The sample was taken after a long period of rain, when water was standing in pools on the field.   If entrapped air in fine porous material were to cause errors, such errors would be expected here.   After the volume was determined, the soil was dried for 6 hours at 96°C. and powdered and the volume determined again.   Each determination was made in five replicates, and each replicate was calculated from six readings.   The results of these 30 determinations are given in table 1.

The known volume of the blocks was used to calculate the volume of the central cylinder to a height of 1 cm.   Measured directly, 100 cc. was equal to 8.60 cm.; and calculated, 8.597 cm.   From the determination of the volumes of the blocks it is possible to find the volume of the blank, which was found to have a mean value of 411.2 cc.   The net volume of the wet soil was calculated to be 90.0 cc., and that of the dry soil, 45.6 cc.   The difference, 44.4 cc., is due to the evaporated water.   The determined loss of evaporation, weighed directly, was 44.0 gm.   No systematic error is indicated, for the error of the volume difference is of the magnitude of 0.8 cc.   The agreement between the two values may therefore be considered complete.

The structural characteristics of the soil were as follows:

Volume percentage of soil	49.2
Volume percentage of water	47 9
Volume percentage of air	2 9
Specific gravity	2.585
Volume weight of dry soil	1.271

[2] According to Maschhaupt and ten Have (12).

MAIN SOURCES OF ERROR

Some unexpectedly high errors were noted. While using the air pycnometer we found two sources of these errors; first, inaccurate readings, and second, the condition of the instrument.

Accurate readings can be expected only if one has had some practice in working with the instrument. A person who is not used to the scales of the instru-

TABLE 1

Soil volume determinations

BLANK TRIAL	62.73-cc. BLOCK	84.43-cc. BLOCK	95.75-cc. BLOCK	GROSS VOLUME OF WET SOIL	GROSS VOLUME OF DRY SOIL
cc.	cc.	cc.	cc.	cc.	cc.

Volume of the soil chamber of the pycnometer

415 0	347 9	324 4	314 8	312 3	361 3
409 9	349 8	326 3	314 7	312 3	363 4
409.3	347 7	325 6	316 3	310 6	363 7
409 6	347 1	325 8	318 3	312 9	363 2
411 0	347 1	325 1	314 5	312 2	360 3
Mean					
411 0	347 9	325 4	315 7	312 1	362 4

Differences between the corrected mean value of the blank and the blocks

0 2	62 8	85 3	95 0	98 7	48 4

Difference between the mean volume and the replicates

+4.0	0 0	−1 0	−0 9	+0 2	−1 1
−1 1	+1 9	+0 9	−1 0	+0 2	+1 0
−1 7	−0 2	+0 2	+0 6	−1 5	+1.3
−1 4	−0 8	+0 4	+2 6	+0 8	+0 8
0 0	−0 8	−0 3	−1 2	+0 1	−2 1

Mean square of the deviations 1.713
Mean error of the single observation . . .. 1 3 cc.

ment makes slight mistakes, which naturally influence the accuracy of the determinations. As the differences in the readings for different samples are small, the readings must be made with due care.

The second source of error causes an excess of about 1 cc. in the result for the first sample of each new series. This is due to deformation of the capillary surface along the dry glass walls and to an uneven temperature distribution in the instrument. The error can be prevented by forcing the water in tube *e* once or twice, before a sample is placed in the instrument, at a pressure higher than will be used in the determination.

SOME RESULTS WITH THE AIR PYCNOMETER

As the air pycnometer was designed principally as a handy field instrument for use in the study of agricultural problems rather than of problems of soil physics, some of the practical results merit discussion.

In an experimental field on a soil consisting of a mixture of sand and peat (*veenkoloniale grond*), large areas of which occur in the Netherlands, the wheat on one plot was attacked by foot rot (*Ophiobolus graminis*). A circular spot of about 1-m. radius was severely damaged. About 3 m. from the damaged spot the wheat was quite sound and might be considered a good crop.

Structure determinations were made on two samples of soil taken in the middle of the diseased spot, on a third sample taken 0.5 m. from the edge of the diseased spot, and on a fourth sample taken in the sound crop. The

TABLE 2

Water and air content of soil samples from wheat field

(Percentage of total volume)

SOIL SAMPLE	DEPTH OF SAMPLE	SATURATED SOIL		SOIL	SOIL IN FIELD CONDITION		SPECIFIC GRAVITY	VOLUME WEIGHT
		Air	Water		Water	Air		
	cm.							
Diseased spot I. . ..	1–6	1 24	48 65	50.1	39.3	10 6	2 22	1 11
	7–12	6 12	63 10	30 8	49 1	20 1	1 94	0 60
Diseased spot II ...	1–6	4 41	48 87	46 7	37 7	15 6	2.32	1 09
	7–12	3 62	54 91	41 5	43 3	15 3	2 13	0 89
Transition zone .	1–6	6 61	49 62	43 8	31 2	25 1	2 22	0 97
	7–12	7 06	57.28	35 7	39 2	25 2	2 16	0 77
Sound spot.	1–6	4 90	51 67	43 4	35 3	21 3	2 33	1 01
	7–12	6 75	54 69	38 6	38 5	22 9	2 25	0 87

ratio soil : water : air was determined. Then the sample in the steel ring was placed in a container in which the water level was kept at a height of 1 or 2 mm. The soil sample became saturated with water as a result of capillary attraction. The part of the volume that still contains air after 24 hours of saturation and 1 hour of draining may be considered as the noncapillary volume or the larger pores.

Table 2 shows the water and the air content of the soil before and after saturation.

The differences in specific gravity are due to uneven distribution of the peat particles in the soil. For instance, in the subsoil of spot I a lump of peat of about half the volume of the sample was found.

Table 2 shows clearly the compact structure of the topsoil. The amount of soil in proportion to the total volume of the sample is highest in the diseased

spots. The volume of the air under field conditions is higher in the sound spot than in the diseased spots, and this relation persists after saturation. The amount of water, taken up by saturation of the soil, is lower in the diseased spots than in the sound spot.

With the measurements obtained, the following rough division in pore size can be made. The air space after saturation is a measure of the large, non-capillary pores. Pores of a somewhat smaller size are filled with air under field conditions and with water after saturation. The smallest pores will be filled with water under field conditions as well as after saturation. There is no boundary between the small and the medium sized pores. The data, here given, depend entirely on the humidity of the soil at the moment the sample was taken. The poreless space in the soil is the volume of the soil material.

If this method of dividing pore spaces is accepted, the conclusion may be drawn that the diseased spots have a larger poreless volume, a larger volume

TABLE 3

Water and air content of soil samples from rye field

(Percentage of total volume)

		STANDING CROP		LODGING CROP	
		Topsoil	Subsoil	Topsoil	Subsoil
Field condition	Air	37 6	21 9	29 5	21 3
	Water	19 5	25 0	24 4	26 5
Saturated soil	Soil	42 9	53 1	46 1	52 2
	Water	40 0	39 0	41 6	39 5
	Air	17 1	7 9	12 3	8 3

of small pores, and a smaller volume of medium sized pores than the sound spot.

Structure measurements were made on a field in which the cereals were lodging. In the middle of an area where rye was lying flat on the earth, a spot was standing upright. Samples were taken from the topsoil, as well as from the subsoil, of both lodging and standing crops, about 1 m. from each other. The results of the determinations are given in table 3.

From the results in table 3 it may be concluded that the volume of pore space in the soil under the lodging crop was smaller than that in the soil under the standing crop, and the volume of air was higher under field conditions as well as after saturation.

If the same division of pore spaces is used as in the previous experiment, it may be concluded that in the topsoil the volume of large and medium sized pores was smaller at the lodging spot than where the crop was standing. The volume of the small pores and of the poreless soil, on the other hand, was smallest where the sound crop grew. The volume of the large pores and of the

poreless soil in the subsoil is about the same in both places, but where the rye was lodging the volume of the small pores is increased at the expense of the medium sized pores.

The two examples given here of the influence of soil structure on the growth of cereals show the interesting features of structure analyses in agricultural problems and indicate the value of an instrument for rapid pore space determination in practical field work.

SUMMARY

The requirements of an instrument to be used for pore space determination in the field are mentioned.

An instrument which seems to meet the requirements of accuracy and usefulness in the field is described.

Two examples are given to show the value of structure analysis by means of pore space determination.

REFERENCES

(1) BURGER, H.   1922   Physikalische Eigenschaften der Wald und Freilandböden.   *Mitt. Schweiz. Centralanst. Forstl. Versuchsw.* 13: 1–219.

(2) BURGER, H.   1924   Die physikalische Bodenuntersuchungen, ins besondere die Methode zur Bestimmung der Luftcapazität   *Proc. Fourth Internatl. Conf. Pedologie* 2: 150–162.

(3) CHRISTENSEN, O.   1930   An index of friability of soils.   *Soil Sci.* 29: 119–135.

(4) COLE, R. C., AND EDLEFSEN, N. E.   1935   A sedimentation tube for analyzing water-stable soil aggregates.   *Soil Sci.* 40: 473–479.

(5) CULPIN, C.   1935   Studies on the relation between cultivation implements, soil structure and the crop.   *Jour. Agr. Sci.* 26: 22–35.

(6) DELILLE, K.   1927   Arbeitsweisen, Arbeitsverfahren und Leistungen beim Zuckerrübenbau im Kreise Marienburg.   *Landw. Jahrb.* 65: 258.

(7) HEATH, O. V. S.   1934   A simple apparatus for measuring the compactness of soil in the field.   *Empire Jour. Exp. Agr.* 2: 205–212.

(8) KEEN, B. A.   1933   Experimental methods for the study of soil cultivation.   *Empire Jour. Exp. Agr.* 1: 97–102.

(9) KEEN, B. A., AND CASHEN, G. H.   1932   Studies in soil cultivation.   *Jour. Agr. Sci.* 22: 126–134.

(10) KUBIENA, W.   1935   Über das Elementargefüge des Bodens.   *Bodenkundl. Forschungen* 4: 380–412.

(11) KUBIENA, W.   1936   Beiträge zur Kenntnis der Gefüges kohärenter Bodenmassen.   *Bodenk. u. Pflanzenernähr.* 2: 1–23.

(12) MASCHHAUPT, J. G., AND HAVE, J TEN   1934   De bepaling van den kalktoestand (verzadigingsgraad) van kleigronden.   *Dept. Binnenland, Zaken en Landb. [Netherlands], Verslag. Landbouwk. Onderzoek. Rykslandbouwproefsta.* 40 (A): 695–775.

(13) MEYENBURG, K. VON   1923   Eine registrierence Zug und Festigkeitssonde.   *Internatl. Mitt. Bodenk.* 13: 201.

(14) NITSCH, W. VON   1936   Der Porengehalt des Ackerbodens Messverfahren und ihre Brauchbarkeit.   *Bodenk. u. Pflanzenernähr.* 1: 110–115.

(15) RUSSELL, J. C.   1934   Scientific research in soil drainage.   *Jour. Agr. Sci.* 26: 544.

(16) SIDERI, D. I.   1936   Soil swelling: I.   *Soil Sci.* 41: 135–151.

(17) SIDERI, D. I. 1936 Soil swelling: II. *Soil Sci.* 41: 275–293.

(18) SIDERI, D. I. 1936 On the formation of structure in soil: I. *Soil Sci.* 42: 381–393.

(19) SIDERI, D. I. 1936 On the formation of structure in soil: II. *Soil Sci.* 42: 461–481.

(20) TIULIN, A. TH. 1928 Some questions on soil structure: II Reprinted from *Results of investigations of the Perm (U. S. S. R.) Agricultural Experiment Station, Division of Agricultural Chemistry*, No. 2.

(21) TORSTENSSON, G., AND ERICSSON, S. 1936 A new method for determining the porosity of the soil *Soil Sci.* 42: 405–417.

(22) WEBB, D. H., JENNINGS, D. S., AND PETERSON, J D. 1936 The effect of replaceable bases on the physical properties of soils with special reference to the effect of replaceable calcium and sodium on index of friability. *Soil Sci.* 41: 13–25.

PLATE 1

The Air Pycnometer

Fig. 1. Pycnometer under pressure.

Fig. 2. Pycnometer opened.   From left to right: the air pycnometer, the ring filled with soil and placed in the ebonite cup, the cover, the ring with screw, and the bicycle pump.

Fig. 2

Fig. 1

479
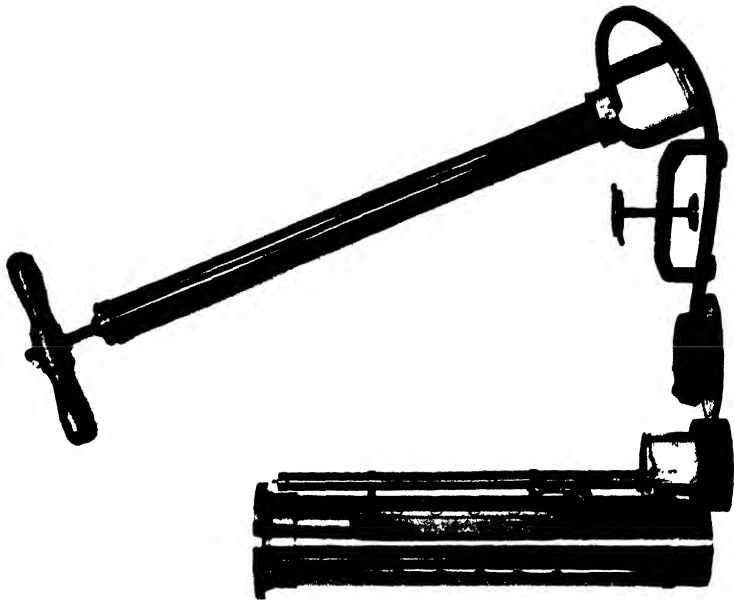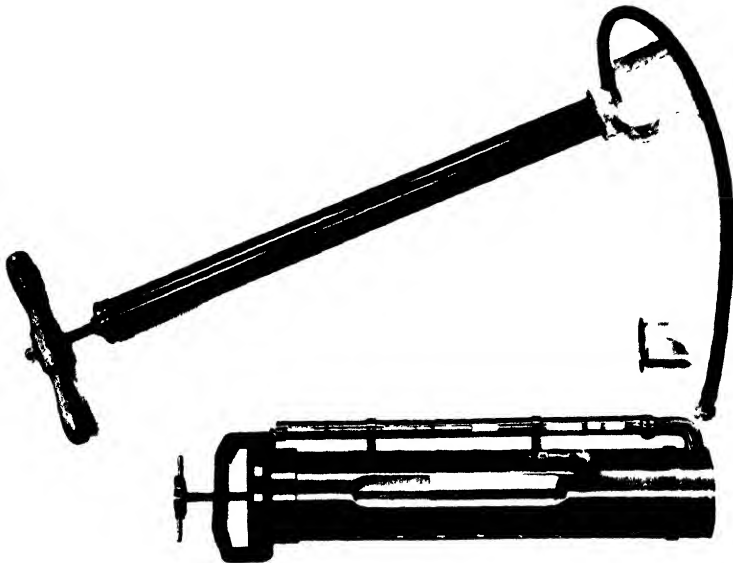
PHYSICAL CHARACTERISTICS OF SOILS: I. NEW METHODS OF MEASUREMENT

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A knowledge of the physical characteristics of soils is essential from the point of view of soil mechanics. Road engineers have developed for some of the more important physical properties a number of tests which have found practical use in earth consolidation. A great many of these tests are adapted to soils in the natural state and thus give measurements under changing conditions. The problem is complicated by the many factors involved in a mixture of sand, silt, and clay of varying composition, and this complication is enhanced by the fact that the nature of clay and possibly of silt may have a profound influence on the physical characteristics of the soil. This paper describes some new methods of measurement which have been found helpful in studying the influence of various factors on the physical properties of soil, an account of which will appear in subsequent parts of this series.

MEASUREMENT OF COHESION

Cohesion is the resistance offered by the soil to any force tending to break it. It is generally measured by applying an increasing force on a rectangular or cylindrical block of soil until the block breaks.[1,2] The disrupting stress is applied by a wedge which is forced in by the help of suitable weights. Alternatively a direct pull is applied to a molded rod of the soil until the rod breaks.

The difficulty of measuring cohesion in dry conglomerates is due to the irregularities inherent in soil make-up and to a lack of control of conditions affecting this property. The results obtained are usually of comparative value only. There is something inherently defective in the measurement of modulus of rupture by forcing a wedge into a dry prism of soil, and reproducible results can be obtained only under strictly controlled conditions.

The apparatus shown in plate 1, figure 1 has certain unusual features and has given uniformly consistent and reproducible results. It consists of a spring balance mounted in a framework which is fitted with a threaded rod that can be moved up or down by the turning of a wheel. To the lower end

[1] HAINES, W. B. 1925 Studies in the physical properties of soils. I. Mechanical properties concerned in cultivation. *Jour. Agr. Sci.* 15: 178.

[2] STAUFFER, L. H. 1927 Measurement of physical characteristics of soils. *Soil. Sci.* 24: 373–379.

of this rod is attached a cylindrical piece of iron about 1.5 cm. in diameter with a hollow coupling so that its flat lower end can be pressed against the test piece without rotating when the threaded rod is lowered.

An important part of the outfit is the brass mold (shown on the left platform of the spring balance) which gives a hemispherical pellet of soil 1 inch in diameter. It consists of two pieces which fit together exactly to form a round cavity 1 inch in diameter and half an inch deep. The soil under test is moistened with water to the sticky point and filled in the cavity, the edges being smoothed with a moistened finger tip. The mold is then split by pulling one portion vertically downward, releasing the pellet. This method of splitting the mold is important because a perfect pellet is not obtained if the pulling is done sideways or with a twisting motion. Once the technique is mastered, soil pellets can be turned out by the dozens in a short time. The soil pellets are air-dried and placed under the threaded rod with the flat side downwards. When the wheel is turned the flat end of the cylindrical piece presses against the pellet with increasing force, indicated on the spring balance, until the pellet breaks. The spring balance needle moves backward slightly when this occurs, but the movement is very slight, and no difficulty is encountered in locating the exact weight at which the pellet breaks. A dozen pellets are generally tested for each soil, and the mean value is taken. Two features of the method are the small quantity of soil required for the test and the uniformity of the stresses and strains involved.

BRINELL HARDNESS TEST

The determination of Brinell hardness number is well known as a standard test for metals, especially steel. A hardened steel ball is pressed into the plane surface of the test piece, by means of a "ball pressure press." The load is measured and removed, and the diameter of the circular impression is determined with the help of a microscope or pocket lens. If D is the diameter of the steel ball, d the measured diameter of the circular imprint, and h the vertical distance through which the steel ball sank, the area of the curved pressure surface is

$$A = Dh = \tfrac{1}{2}\pi D(D - \sqrt{D^2 - d^2})$$

Then if W is the applied load, we have

$$H_b = \frac{W}{A}$$

and H_b is called the Brinell hardness number and is usually expressed in kilos per square millimeter. The following values for Brinell hardness number are given for illustration and for comparison with soils:

Vulcanized fiber.. 7–16 kgm./sq. mm.
Zinc... 50   kgm./sq. mm.
Copper 60   kgm./sq. mm.
Steel.. 150–300 kgm./sq. mm.

The application of this test to soils was pointed out by Haines,[3] but certain difficulties were encountered which could not be overcome.

The same apparatus is used as for soil cohesion, with the modification that the wheel is marked into 200 divisions and an upright brass piece indicates the complete turns of the wheel; thus any fraction of this turn can be indicated. The soil under test is brought to the sticky point, filled in a brass ring 1.8 cm. wide and 3.5 cm. in diameter, and allowed to dry. The soil block, on drying, falls out as a result of shrinkage. It is then tightly held in a hose clamp. The clamp containing the soil, together with the steel ball, is shown on the right platform of the spring balance (pl. 1, fig. 1).

The steel ball, which has a diameter of 8 mm., is soldered to an iron rod which can be attached to the lower end of the screw in the cohesion apparatus, in such a way that when the wheel is rotated, the ball moves downward but does not turn.

The zero reading of the instrument is taken by pressing the steel ball against the spring balance and giving one complete turn to the wheel. This moves the ball 4.8 mm. on the instrument used (this distance will vary according to the pitch of the screw), and the spring balance indicates a certain weight (31.5 kgm. in this case). The pressure is then released, the clamped soil block is placed on the balance, the steel ball is brought against it, and the wheel is again given one turn. A portion of the steel ball sinks into the soil, and the spring balance records a smaller weight. The wheel is then gradually rotated further until the spring balance records the same weight as it did without the soil block. This extra turn represents the distance (h) through which the steel ball has sunk into the soil block. As one full turn indicates a distance of 4.8 mm., the depth of the depression can be measured directly with an accuracy of approximately 0.02 mm. The accuracy could be easily doubled, but this is not necessary for soil work.

We have seen that the Brinell number $H_b = \dfrac{W}{A}$. As W is constant in all tests and A is equal to Dh when D is the diameter of the steel ball and h is the depth of the depression (given by the extra turn), we have

$$H_b = \frac{W}{D} \cdot \frac{1}{h} = \frac{k}{h}$$

For a particular instrument, k is determined once and H_b values are obtained by simply dividing a constant factor by h. The value of k for the instrument used was 1.21.

MEASUREMENT OF EROSION

Erosion is the disintegeration of the soil under the impact of water, which may be in the form of a jet, a stream, or drops. Resistance to erosion is an important property of soil, a study of which is of great importance in agricul-

[3] See footnote 1.

ture and forestry and also in determining the durability of mud plaster in areas of moderate rainfall where this stuff is used for protecting mud houses. Hitherto the problem has been studied chiefly in soil *in situ*, and the author is not aware of any previous attempts to measure this property in the laboratory on prepared soil blocks.

A simple apparatus was devised for studying the influence of various factors on erosion. This, as shown in plate 1, figure 2, consists of a vertical brass tube, 1 inch in diameter, having two side tubes near the bottom, one for connection to the water tap and the other ending in a narrow jet, which is screwed on and can be changed when desired. A brass cup is attached to the vertical tube just below the open upper end to catch the overflow water, which is drained

TABLE 1

Physical characteristics of various soils

SOIL NUMBER	CLAY	COHESION	BRINELL NUMBER	EROSION TIME
	per cent	*kgm.*	*kgm./sq.mm.*	*minutes*
5	4 5	6.1	0 36	11 2
6	10 5	12 3	1 26	5 6
8	20 2	26 3	1 92	54 8
9	5.4	4 5	0 48	3 6
10	16 9	10 4	1 13	4 0
11	7 5	9 5	1 26	7 3
12	13 7	11 3	1 18	9 3
13	7 2	8.2	1 20	4 3
14	12 7	13 2	1 36	8 6
15	22 8	17 8	2 24	38 1
16	6.6	10 0	1 38	8 1
17	9 28	11.8	0 88	6 6
18	10 5	11 8	1 13	9.6
19	27 7	30 5	2 78	60.8
21	35 4	38 1	2 76	39 7
22	17.2	23 3	1 98	6 4
23	35 9	41.8	2 57	20 0
24	42 9	49 5	2 55	29 9

away through an outlet at the bottom of the cup. The brass tube is mounted on a platform which can be moved in two directions and the position of which can be adjusted anywhere on the horizontal plain by turning of the screws.

When the brass tube is connected to a reservoir of water so that it just overflows at the top, a perfectly uniform jet of water is obtained which breaks into small droplets as it falls. These droplets are made to impinge on a dumb-bell-shaped soil block resting on two glass rods. The erosion time is reckoned from the start until the soil block is so worn that it falls.

The values of the erosion time depend on the diameter of the jet, the head of water, and the size of the soil blocks, all of which are arbitrarily fixed. The head of water, in this case, was 100 cm., reckoned from the tip of the jet, which

had an opening 1 mm. in diameter; and the dumb-bell-shaped soil blocks were 7.5 cm. from end to end, 2.5 cm. wide at the middle and 4.5 centimeters at the greatest breadth, and 2.25 cm. thick. These dimensions are of no special advantage except that they are the dimensions of standard molds available in connection with cement-testing machines. Rectangular or circular prisms of soil might be found equally satisfactory or even more satisfactory.

This apparatus can be used also for testing the erosion of a soil block in the natural state. For this purpose a circular block is separated from the rest of the soil by ramming into the soil a shallow iron cylinder slit in one place to provide for the run-off. The soil block can be removed in the cylinder, or the cylinder can be left in and the soil tested *in situ* by adjusting the jet so that the drops fall uniformly on the whole area. The apparatus showed appreciable reduction in erosion of soil *in situ* due to a cover crop like grass. Of course the erosion is measured by collecting the run-off and determining the material eroded in unit time.

RELATIONSHIP BETWEEN MEASUREMENTS

In order to illustrate the use of the different apparatus and to show the relationship between the various measurements, the cohesion values, the Brinell numbers, and the erosion times of 18 soils, together with their clay contents, are given in table 1. The influence of clay is apparent in all cases, though erosion does not seem to depend largely on the clay content. This is illustrated by the following significant correlation coefficients between the various measurements:

Clay percentages and cohesion values 0 97
Clay percentages and Brinell numbers 0 90
Clay percentages and erosion times 0 64
Cohesion values and Brinell numbers 0 89
Cohesion values and erosion times...................................... 0 63
Brinell numbers and erosion times................. .... 0 74

SUMMARY

Three new apparatuses for measuring physical characteristics of soil are described. One gives the cohesion values; one, the Brinell numbers; and one, the erosion times. Results with a few typical soils are given by way of illustration.

PLATE 1

APPARATUS FOR MEASURING SOME PHYSICAL CHARACTERISTICS OF SOILS

FIG. 1. Apparatus for measuring soil cohesion and Brinell hardness.
FIG. 2. Apparatus for measuring soil erosion.

FIG. 2

FIG. 1

AUTHOR INDEX

SUBJECT INDEX

Microorganisms, abundance as influenced by organic matter, 433

Minor elements, use in North Carolina agriculture, 251-263

Moisture—
content of soils, method of determining 377
equivalent of soils, 69
properties of clay, 25
sampling for soil, 24

Molybdenum, response of barley to, 107

Mull, composition, 232

Neubauer method for available potash, 267

Nickel, response of barley to, 107

Nitrates—
biological vs. photochemical formation, 444
cellulose decomposition and, 393
fixation in soils, 371
formation in soil, 361-375
in organic remains of forest litter, 232
in soil as affected by time of application of nitrogen, 294
irradiation effect on formation of, 441

Nitrification—
as affected by—
bentonite, 321
drying, 368
humus, 321
sunlight, 365
in ammonium culture solutions, 95
in cropped and uncropped soils, 148

Nitrogen—
amide, in soybeans, 207
amino, in soybeans, 207
distribution in soil profile, 73
fertilizer, effect on wheat when added in fractions, 293
fixation—
as affected by bentonite and humus, 321
hypotheses on, 208
relation to soybean metabolism, 203
fractions in cotton plants, 220
in forest litter, 232
metabolism of soybeans, 203-215
nutrition of barley, 91
source, as a factor in cellulose decomposition, 387
transformation from rye and clover, 431

Nutrients—
in organic layers of forest soils, 231
major, in relation to minor elements, 256

Organic—
carbon, titration method for determining, 141
colloids—
effect on migration velocity of soil colloids, 349
in reaction with inorganic colloids, 331
matter—
base exchange capacity, nitrogen, calcium, potassium, phosphorus content of, in relation to growth of spruce, 233
changes in, as a result of cropping, 139
destruction, method, 87, 124
distribution in soil profile, 74
in cropped and uncropped soils, 143
quality, 148
relation to potassium fixation, 274
removal for exchange studies, 123
removal for mechanical analyses, 68, 87
matter, effect on—
cellulose decomposing organisms, 436
phosphate fixation, 279
remains, productivity of, 232
soils, see Soils organic

Oxygen supply, relation to nitrogen nutrition of barley, 91

Pears—
arsenic assimilation by, 44
copper assimilation by, 54
lead assimilation by, 49

Phosphates—
calcined, efficiency, 175-183
fixation in soils, 277-291
relation in soil to weight of roots and shoots, 185-202
rock, from several sources, composition of, 177

Phosphorus—
absorption by plants, 179
available, in forest, 232

Plant—
material, composition, 427
nutrition—
minor elements in, 21
soil properties affecting, 23

Plants—
metabolic activities as influenced by nitrogen carriers, 110
phosphoric acid in, grown with different sources of phosphorus, 179